THE ELECTRICAL CONDUCTIVITY OF AQUEOUS SOIL SUSPENSIONS AS A MEASURE OF SOIL FERTILITY.

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(With Five Text-figures.)

INTRODUCTION.

IN a previous paper one of the authors[6] confirmed the observations of Atkins[1] that there was a rapid increase in the electrical conductivity of aqueous extracts of soil as the extraction was prolonged in the case of fertile soils, while there was little or no increase in the case of infertile soils. It was further shown that, for soils collected at the same time from adjacent plots, the increases in conductivity after 8 to 10 days corresponded with the crop yields from these plots. From this it seemed probable that if samples of soil were taken at intervals and at known dates from plots under continuous cultivation, the decrease in fertility would be shown by corresponding decreases in the electrical conductivity. In fact, the other author[8], who had found that the specific conductivity of a soil extract is a measure of the fertility of the soil, obtained indications of decreases in conductivity in Nigerian soils under cultivation; the results were not conclusive, probably because the period over which the soil samples were collected was too short.

The soil samples that have been taken at intervals from the Rothamsted classical plots and carefully preserved in bottles are probably the most valuable material for testing the relationship between electrical conductivity and soil fertility. It was, therefore, decided to measure the "initial conductivity," *i.e.* specific conductivity of a 1 to 5 aqueous suspension determined at 25° C. in mhos (*i.e.* reciprocal ohms) and the "7 days' increase," *i.e.* the rise in specific conductivity of the same aqueous suspension after standing for 7 days in the thermostat at 25° C.

<center>SOIL SAMPLES USED.</center>

Stored soil samples from the following Rothamsted plots were examined:

(1) Broadbalk Plot 2 B   Manured with 14 tons of dung per acre.
(2) Broadbalk Plot 3   Unmanured.
(3) Broadbalk Plot 7   Manured with 412 lb. of sulphate of ammonia, 3½ cwt. of super-phosphate, 200 lb. of sulphate of potash, 100 lb. of sulphate of soda and 100 lb. of sulphate of magnesia per acre.
(4) Hoosfield Plot 1–O   Unmanured since 1852 and under barley every year.

All the Broadbalk plots, except Plot 3, receive the same manure annually, and have been under wheat since 1843. Previous to that year the plots were all treated alike. The crops grown and the manures applied for the previous 5 years were turnips with farmyard manure in 1839, and subsequently barley, peas, wheat and oats without manure.

<center>EFFECT OF STORAGE ON CONDUCTIVITY.</center>

Since most of the soil samples were stored for many years, differences in the measurements, if any, might be due to (i) seasonal differences, since all the samples were not collected at the same time of the year, or (ii) differences in the period of storage. The first of these will be fully dealt with by the first author[7]: it will be shown that there is no significant seasonal variation in the measurements for the Rothamsted soil from the unmanured plot, but the measurements for the manured plots are affected temporarily by the application of manure and the growth of the crop. Consequently the main conclusion is drawn from the measurements on unmanured soil.

With regard to the second factor there was, of course, no information whether prolonged storage might result in some slow changes likely to affect the measurements. However, as the soil samples had always been stored in the air-dry condition, in air-tight bottles, it appeared reasonable to assume that any such changes would occur mainly during the initial stages of storage. The following experiments were, therefore, conducted to study the influence of the initial storage on the measurements. The total duration was slightly over nine months. Composite samples of soil, each of which was a mixture of three separate holes (0–9 in.), were taken from three Broadbalk plots, 2 B, 3 and 7. They were dried in the air at room temperature for 8 to 10 days, passed through a 1 mm. sieve and stored in bottles. The conductivities of the aqueous suspensions of the air-dry samples thus stored were determined in duplicate as follows: 100 c.c. of conductivity water were added to 21·2 gm. of air-dry soil (*i.e.*

20 gm. of oven-dry soil) in a bottle and the mixture was shaken for 1 hour in an end-over-end shaking machine and, after subsequent standing for 40–45 minutes in the thermostat at 25° C., its conductivity was measured. The increase in conductivity after 7 days' standing was
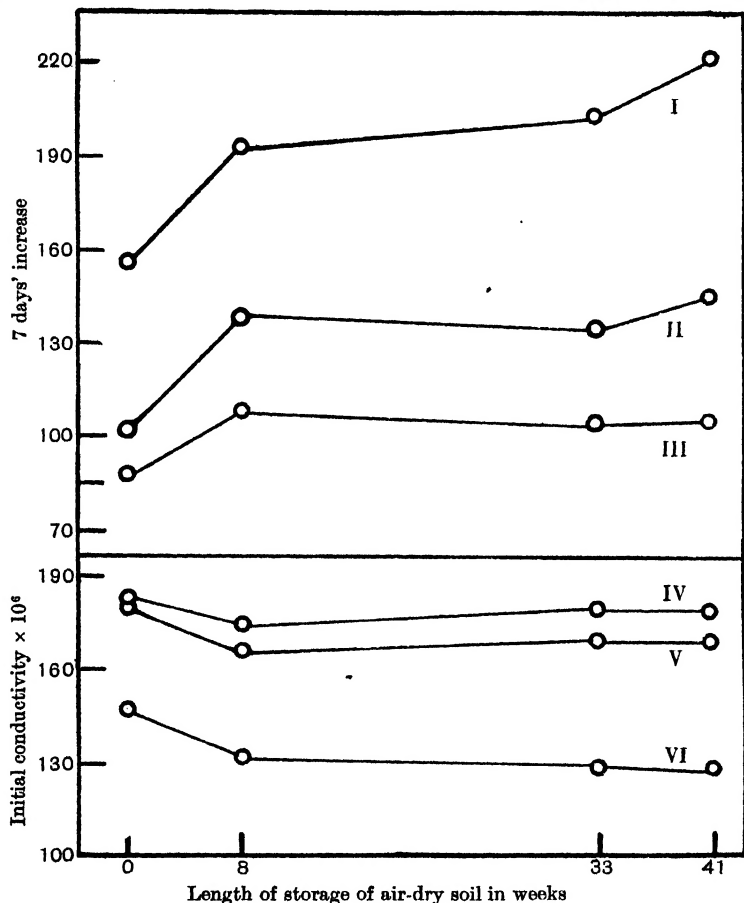

Fig. 1. Broadbalk field.

Curves I and IV, Plot 2. Farmyard manure.
    „   II and V, Plot 7. Minerals.
    „   III and VI, Plot 3. No manure.

also measured. The technique will be fully described by the first author[7]. The results are given in Fig. 1.

The initial conductivity measurements for the three Broadbalk samples (Fig. 1) decreased slightly during the first few weeks' storage,

but remained remarkably constant from the eighth week till the end of the experiment. It is evident therefore that prolonged storage has very little effect on the initial conductivity of air-dry soil. In the case of unmanured soil or that treated with inorganic fertilisers the 7 days' increase became larger with continued storage, but attained a fairly constant maximum value in the course of the first 2 to 4 months. On the other hand, in the case of the dunged soil, the maximum was not reached even after storing for 41 weeks. But as the value was increased by 37 units after the first 8 weeks' storage, while on continued storage for a further period of 33 weeks it was increased by 28 units only, it may be expected that, on continuing the experiment further, a maximum value would have been reached, probably within a few months.

Parallel experiments were also made on samples taken from another field; they gave results closely similar to the Broadbalk samples.

In view of the above observation it is assumed that, since the old soil samples forming the subject of the present investigation have been stored for many years, they have already attained the maximum values and are therefore comparable.

METHODS.

In 1927 fresh soil samples were collected from the Broadbalk plots, 2B, 3 and 7, and Hoosfield plot, 1–O, dried in air for 10–12 days, passed through 1 mm. sieve and then preserved in corked bottles for over 1 year so as to make them comparable with the other old stored samples from these plots. Since most of the other samples were available in very small quantities, the following modification in the preparation of the soil suspensions was adopted: 10 gm. of air-dry soil were taken in an 8 in. Pyrex test-tube to which 50 c.c. of conductivity water were added. The mouth of the tube was closed by means of a paraffined cork, and the mixture was thoroughly shaken for half a minute. It was then allowed to stand in the thermostat at 25° C. and, after 24 hours, the initial conductivity was determined, using a dip-electrode. The latter was withdrawn and the tube containing the suspension was left in the thermostat for 8 days. On the fifth day the suspension was shaken once for half a minute, and on the eighth day the final conductivity was measured, using the same dip-electrode. The difference between the final and initial conductivity gave the "7 days' increase."

Broadbalk Plot 3.

In Fig. 2 the measurements of initial conductivity and 7 days' increase are given for the stored samples from this plot, together with the

crop yields. The latter are given in two different forms. One (Curve III) is the actual yield of the particular year for which the stored sample was available, while the other (Curve IV) is the average yield for three consecutive years, viz. the particular year of sampling and the years immediately preceding and following it.
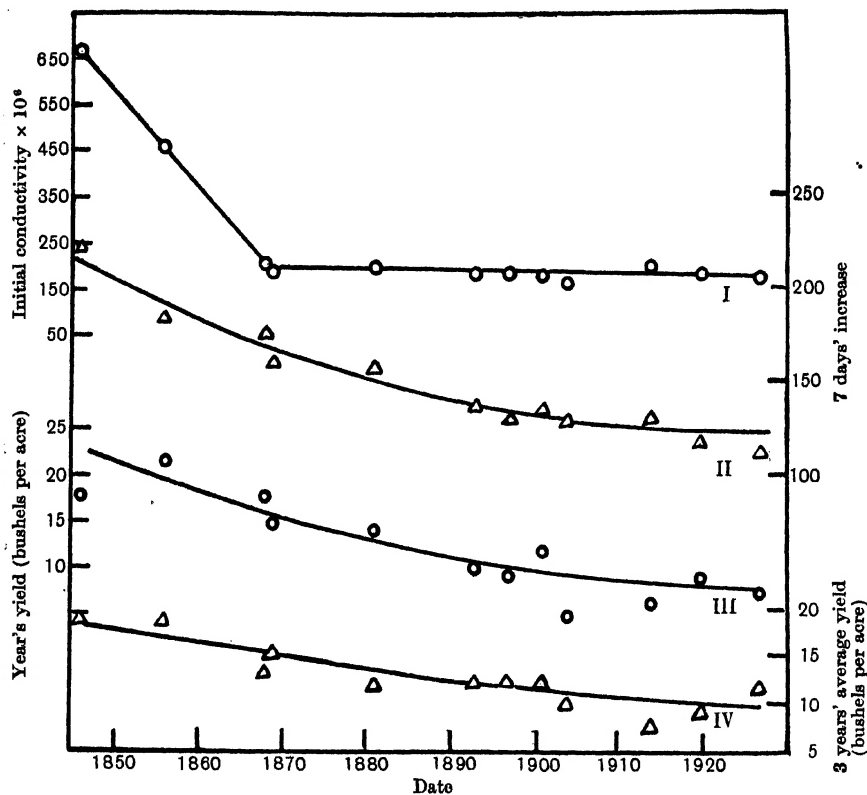
Fig. 2. Broadbalk Plot 3. No manure.

I. Initial conductivity of air-dry soil samples collected during the years shown.
II. Seven days' increase of air-dry soil samples collected during the years shown.
III. Yield of wheat in bushels per acre for the years shown.
IV. Average yield of wheat in bushels per acre for three years, viz. the year shown and the year preceding and following it.

Under continuous cropping the initial conductivity (Curve I) of Plot 3 steadily decreased from the year 1846 to 1869. After this date sensibly constant values are given. It has been observed by the senior author(7) that the initial conductivity of a plot, which increases on the

application of manures, comes down to the value previous to manuring in the course of a few months. Consequently, as the above plot was not manured after 1839, the high initial conductivities of the samples of 1846 and 1856 were probably not due to the residual effect of previous manuring. The soluble salt content of the plot, which was therefore normally high up to 1856, was gradually brought down under continuous cropping to a certain low value in 1869, and afterwards changed very little over a period of 58 years. This shows that the crop can deplete the soil of its soluble salts only up to a certain point. It is interesting to note that Burd and Martin[2], while studying changes in the soil solution, observed that in soils (contained in vessels) which have been cropped for some years, the initial concentration of the solution in any given growing season returns to its original magnitude by the beginning of the following season.

Fig. 2 shows also that there is only a very general relation between the initial conductivity and crop yield, viz. the higher initial conductivities before 1868 are associated with higher yields. The distinct tendency for a gradual decrease in the yield of wheat under continuous cropping after 1868 (as seen in the 1-year yield figures) is not reflected in the initial conductivity. On the other hand, a close relationship is evident between the 7 days' increase figure and that for the 1 year's crop (Curves II and III), both decreasing with time under continuous cropping. The correlation coefficient between the two sets of values is +0·859.

Hoosfield Plot 1–O.

The results are given in Fig. 3. The first sample from this plot was not taken until after 30 years of continuous cropping without manure, hence much of the information regarding the relationship between the measurements of conductivity and crop yield is probably lost, since it has been shown above that the changes both in the measurement and the yield of Broadbalk Plot 3 are very small after a period of 30 years' cropping. With the exception of the two high measurements for the samples of 1889 and 1913, the initial conductivity (Curve I) for this plot shows a regular slow decrease. It is difficult to explain the reason for the two high measurements mentioned above. The plot was fallowed in 1912, but whether this was in any way connected with the increased measurement in 1913 is not known. But it may be mentioned that continued fallowing of Rothamsted plots was not found[7] to affect the measurements.

The yield of this plot was found to fluctuate very greatly, but the 3 years' average figures show a gradual falling off in the yield. On the whole there is a general parallelism between this set of figures and that
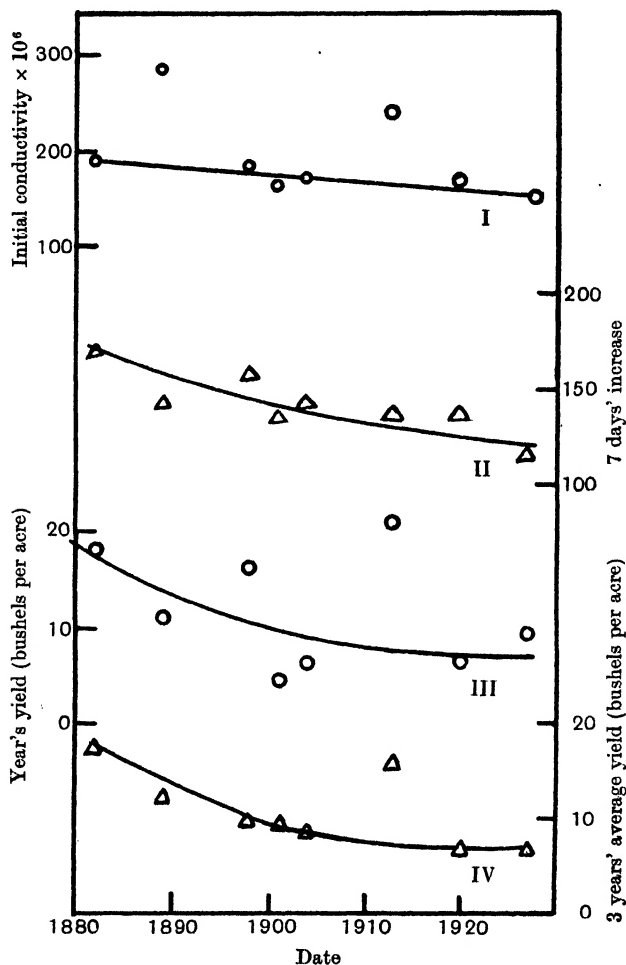
Fig. 3. Hoosfield Plot 1-O. No manure. For explanation of numbering see Fig. 2.

for the 7 days' increase. The high yield obtained in 1913 can be attributed to the previous fallowing as shown below.

Year	1910	1911	1912	1913	1914	1915
Yield of barley in bushels per acre	9·9	4·9	Fallowed	21·1	10·4	8·5

Broadbalk Plots 7 *and* 2B.

The results for these two plots are given in Figs. 4 and 5. For Plot 2B the earliest sample available was that of 1868, and for Plot 7 that of 1881. Here again Curve II in each of the two figures shows a
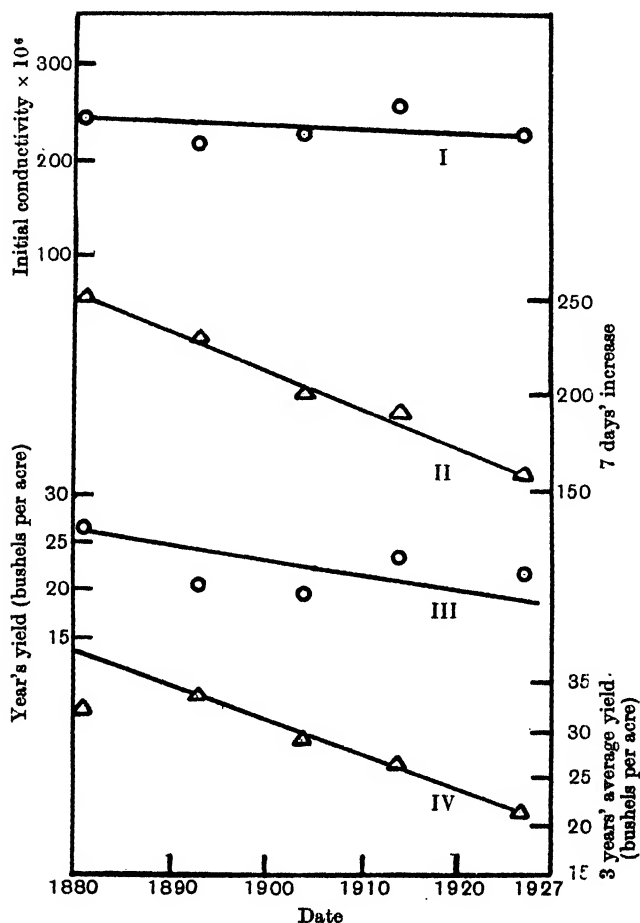
Fig. 4. Broadbalk Plot 7. Inorganic manures. For explanation of numbering see Fig. 2.

distinct tendency in the 7 days' increase to fall off, but the number of stored samples available for examination is too small to warrant any detailed discussion. One fact, however, is fairly obvious; by yearly application of manure the 7 days' increase of both plots was maintained throughout at a higher level than that of the unmanured plot (Curve II,

Fig. 2). Also this difference in the levels was greater when dung was applied (Plot 2 B) than when inorganic fertilisers only were used (Plot 7).

The results show that the initial conductivities of the earliest samples
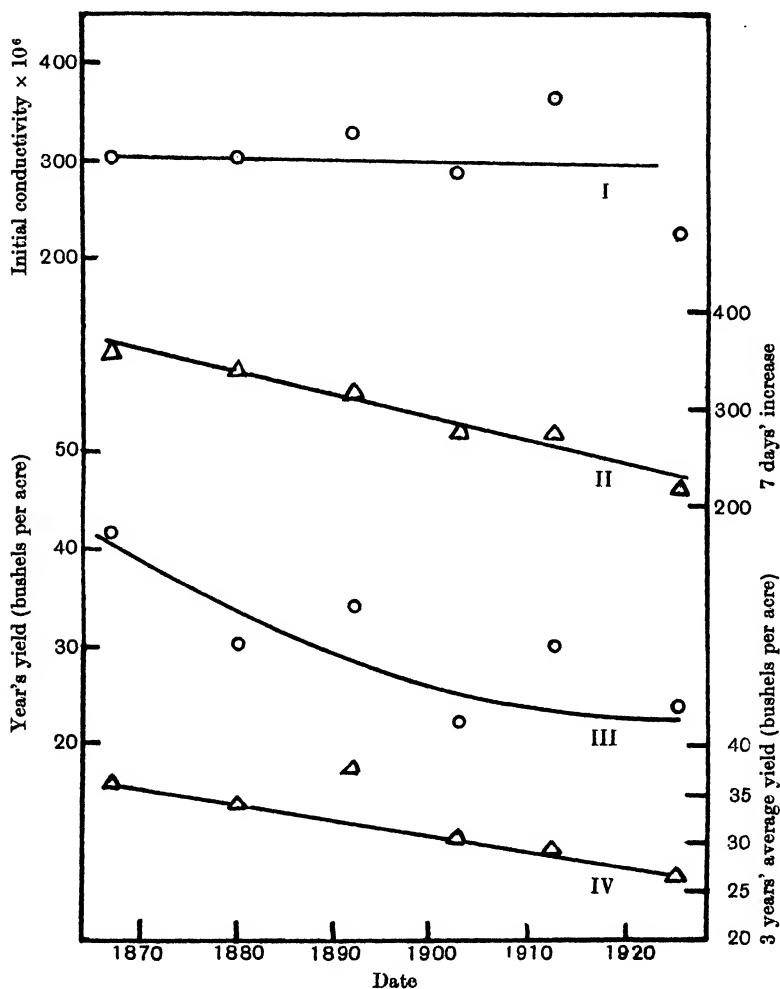
Fig. 5. Broadbalk Plot 2 B. Farmyard manure. For explanation of numbering see Fig. 2.

from Plots 2 B and 7 (1868 and 1881 respectively) are lower than the earliest samples from the unmanured plot (1846 and 1856). Since all the plots received exactly the same treatment prior to 1846, it is reasonable to conclude that, in spite of the manures applied, the normal conduc-

tivities fell between 1846 and 1869 in the case of Plot 2B and between 1846 and 1881 in the case of Plot 7. After this date the conductivities remained fairly constant for any one plot. It is also of interest to note that this constant value for plots 2B, 7 and 3 are in descending order of magnitude. It appears, therefore, from the foregoing results that, under continuous cropping, the normal initial conductivity of a soil, whether uniformly manured or unmanured, decreases to a low value, which afterwards remains or tends to remain fairly constant over a large number of years, the minimum value depending slightly on the kind and probably on the quantity of manure applied.

BROADBALK WILDERNESS AND ADJOINING GRASS LAND.

That fertility increases when land is in the state of permanent grass or under natural vegetation is well known. The purpose of the present experiment was to see if such gain in the fertility is reflected in the measurements. In 1882 a portion of the upper end of Broadbalk field was not harvested, but was fenced and allowed to run wild. A more detailed description of this area, known as Broadbalk Wilderness, together with figures for nitrogen that accumulated in 20 years showing increased fertility, is given by Hall(4). There is also a strip of land between the lower end of the Broadbalk plots and the brick trench for collecting pipe drainage, which has been under a thick growth of grass for many years. In 1928 separate samples were taken from the portions of the Wilderness and of the grassland which was previously included in Plot 3. Measurements were made on these samples after they had been dried in air. The results, together with the measurements for Broadbalk Plots 3 and 2B for comparison, are given in Table I below.

Table I.

Plot no.	Sample collected in	Initial conductivity $\times 10^6$	7 days' increase $\times 10^6$
3	1881	197	157
Wilderness	1928	338	295
3	1894	179	136
Grassland	1928	277	223
2B*	—	305	293

* Mean values of all the samples between 1868–1927.

If the soil conditions of the Broadbalk Wilderness in 1882 and those of the grassland in 1896 are assumed to be very nearly the same as those of Plot 3 in 1881 and 1894 respectively, the above results show that

the increased fertility which the Wilderness and the grassland have gained by 1928 was reflected also in the measurements. It is interesting also to find that the mean measurements for Broadbalk Plot 2B approximate closely to the measurements for the Wilderness and the grassland. This shows that by yearly application of dung the fertility of the soil can be maintained near that of a soil under natural vegetation.

RELATION OF 7 DAYS' INCREASE TO YIELD OF CROP.

It has been shown that under continuous cropping the 7 days' increase decreases steadily, and also the crop yield, and that there is a distinct parallelism between them. The significantly high positive correlation coefficient between the two curves, II and III in Fig. 2 ($+0.859$), is undoubtedly the best indication of the parallelism between the two series of values. When, however, the time factor is eliminated from the two series, the partial correlation coefficient between them is found to be insignificant, viz. $+0.061$. The result is not surprising, since the variation in the crop yield is the resultant of three types of variation as distinguished by Fisher[3]; these are (i) annual variation, (ii) steady diminution due to deterioration of the soils, and (iii) slow changes other than steady diminution. The main trend of the curve for crop yield is due to the progressive changes taking place in the soil, whereas the annual fluctuations are doubtless due to climatic factors both in the case of wheat[3] and barley[5]. Evidence has been obtained by the senior author[7] that these climatic factors have little effect on the 7 days' increase of arable Rothamsted soils, and consequently a high partial correlation between this measurement and crop yield would not be expected. On the other hand, the high positive correlation obtained between the two quantities shows that the 7 days' increase is a very satisfactory indication of the changes taking place in the fertility of the soil itself.

The results obtained in this paper, therefore, lead to the conclusion that, under continuous cropping, there is a steady diminution, at first rapid but afterwards slow, in the 7 days' increase of a soil, particularly if the soil is unmanured, and that this gradual diminution is correlated with a gradual fall in the crop yield.

SUMMARY.

Measurements have been made of the electrical conductivity of aqueous suspensions of stored soil samples taken at intervals of several years from four of the Rothamsted classical plots bearing the same crop every year. The results show that under continuous cropping:

1. The initial conductivity (and therefore the soluble salt content) of an unmanured soil decreases steadily to a minimum value, which then remains fairly constant over a long period of years. There is reason to believe that, under the same conditions, the normal initial conductivity of a continuously manured plot (excluding the temporary increased measurements due to application of manure) decreases similarly to a fairly constant minimum value, which is slightly greater than that of an adjacent unmanured soil depending on the kind and probably on the quantity of manure applied.

2. The 7 days' increase of both unmanured and manured soil decreases progressively. In the case of an unmanured soil for which earlier samples were available, the 7 days' increase is found to decrease comparatively rapidly during the first few years of continuous cropping.

3. The 7 days' increase of a soil manured every year is maintained throughout at a higher level than that of an adjacent unmanured soil. The difference in the levels is greater when dung is applied than when inorganic fertilisers only are used.

4. There is a high positive correlation ($+0.859$) between the 7 days' increase of stored samples for various years and the crop yield for those years. But when a partial correlation is calculated, eliminating the time factor, the value is found to be insignificant ($+0.061$).

Evidence is also given that, on allowing a soil in a low state of fertility due to continued cropping to run wild, or on leaving it under grass, there is a marked increase both in its initial conductivity and 7 days' increase. This result is thus in accordance with the well-known fact that a soil left to either of the above two conditions gains in fertility.

During a preliminary investigation it was observed that, on prolonged storage of an air-dry soil, the initial conductivity is not altered significantly, but the 7 days' increase rises rapidly in the course of a few months to a fairly constant maximum value. This value, and the time required to attain it, both depend on the previous manurial treatment of the soil.

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THE IMPORTANCE OF THE SHAPE OF PLOTS IN FIELD EXPERIMENTATION.

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(With Four Text-figures.)

INTRODUCTORY.

IN the course of the last 20 or 25 years it has been established clearly that agricultural experiments carried out under field conditions are subject to an appreciable error, due chiefly to soil heterogeneity. In order to avoid the effect of this disturbing factor, which greatly affects the reliability of data given by field trials, two questions had to be carefully considered: (1) how to secure greater uniformity in soil conditions; and (2) what is the best method of getting a criterion of the accuracy of the experiment and an adequate interpretation of the results obtained?

In connection with the first question a good deal of work has been done by several workers, whose attention was concentrated chiefly on the importance of the size and the shape of the plots, their arrangement and orientation, the use of artificial plots, the practice of replication, the selection of a uniform experimental field, the border effect and competition, and on some other questions of secondary consequence.

Nevertheless, although the work so far accomplished in this direction made a great contribution towards the reduction of the error attached to field experiments, it is generally recognised that the results obtained have not been quite satisfactory. In spite of the greatest attention and the most meticulous precautions we may take in order to secure uniformity of experimental conditions, it is almost certain that the yields of two near-by plots similarly treated will be anything but equal.

There is no doubt that this great difficulty in overcoming soil heterogeneity in any of the above direct ways led in recent years to the general application of statistics in yield trials, thus giving rise to the development of new methods for tackling the puzzling question of soil heterogeneity. Instead of trying actually to eliminate the differences of fertility existing amongst the parts of the same field, it is intended to minimise their effect by securing the lowest possible and presumably the most

accurate determination of the experimental error, thus increasing the significance of the experiment. In this connection a few methods have in recent years been devised, of which Fisher's "Analysis of Variance" seems by far the most important.

It must not be lost sight of, however, that, in spite of the fascination which an accurate statistical analysis of the agricultural problems carries with it, soil heterogeneity cannot be settled definitely in this way. After all, what we are looking for in carrying out experiments in the field is not just the determination of the error, but a clear and definite answer whether the variety or treatment A is better than the variety or treatment B. Now supposing that with regard to yield the difference between A and B is 15 per cent. of their mean and its standard error ±10 per cent., it is well known that from the statistical point of view this difference is not significant. From the farmer's point of view, however, a difference of 15 per cent. is certainly very significant, and if we take into account the production of a whole country, 15 per cent. is of course of great economic and probably social or other importance. Therefore, no matter how accurate and appropriate the statistical methods we are using, it is impossible to go beyond the limits set by the experimental data themselves, and as long as they exhibit a great divergence for plots similarly treated, it is obvious that the problem of field experimentation needs further investigation.

PREVIOUS WORK.

When studying the literature concerning the various methods of plot technique one can hardly fail to notice that the effect of the shape of plot has been more or less disregarded. Although, incidentally, a good many workers paid attention to this particular question, none of them undertook its thorough investigation, the shape of the plots being in the majority of cases considered as a question of secondary importance. Consequently it is not surprising that the conclusions arrived at by different workers are so contradictory in this respect. Mercer and Hall(10), for instance, discussing their mangold experiment, state that "little can be deduced as to any superiority of long and narrow plots over square ones." Later on Lyon(9) came to the same conclusion when check plots were not used. With checks, however, he found an advantage with long narrow plots. Kiesselbach(6) proved that the variability with oblong plots is smaller than with square ones, while Day(1) pointed out that this is true only if the length of the plot lies along the direction of the greater change of soil fertility, otherwise square plots were preferable.

From the above and other similar cases, some of which will be considered later, it is evident that the effect of the shape of the plots has not so far been elucidated. What is needed is a satisfactory explanation of the results previously obtained, and an adequate investigation on the possibility of reducing soil heterogeneity by the use of a certain shape of plot.

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THEORETICAL CONSIDERATIONS ON THE EFFECT OF
THE SHAPE OF PLOTS.

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Generally speaking, soil heterogeneity can be conceived under two main forms: either as patches of ground of higher or lower fertility scattered all over the field more or less at random, or as gradual change of productivity from the one side of the field to the other.
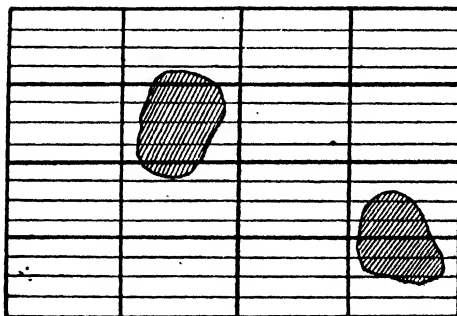
Fig. 1. Random patches affecting long and square plots differently.

(a) *Patched heterogeneity.* Considering the patched type of heterogeneity first, one can easily see that in this instance the use of square, or long and narrow, or any other shape of plot is immaterial from a theoretical standpoint. Since the patches are distributed at random amongst the plots of the field, it does not make any difference what is the shape of the plots, so long as their size is the same. Moreover, as the probability of the occurrence of these patches is governed by the laws of chance, it is not difficult to avoid their effect by adopting a sufficiently large number of replications and making use of their averages. If it is required to express this fact in mathematical terms, one can say that the odds of a plot containing 0, 1, 2, 3 ... n patches are given by the expansion of the binomial $(p+q)^n$, where n denotes the total number of patches in the field, p is the reciprocal of the number of plots into which the field has been divided, and $q = 1 - p$.

In fact, long narrow plots seem far preferable to square ones. A patch might sometimes affect the whole plot if square plots have been adopted, whereas with long plots whose width is 25, 50 or even 100 times less than their length, such a patch is likely to affect a great number of consecutive plots and only a small proportion of their area. Fig. 1 illustrates this point fairly well. Of course a long narrow patch might just fit a long plot, thus hopelessly vitiating the results of the experiment; it must be admitted, however, that the chance of such an event occurring is too remote to be taken into account for practical purposes.
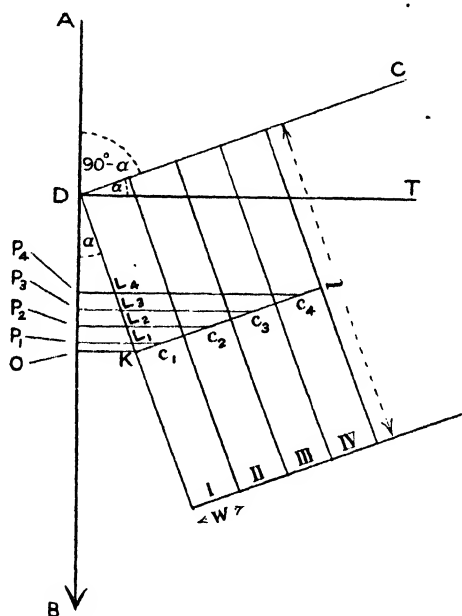
Fig. 2. Uniform change of fertility.

Moreover, in agricultural experiments, the number of observations rarely, if ever, is sufficiently large to allow full play to the laws of chance; therefore the use of long plots constitutes the only means of reducing the effect of patched heterogeneity.

(b) *Gradual change of fertility*. With regard to a gradual change in soil fertility a hypothetical case is first considered where the productivity of the field increases uniformly from A to B (Fig. 2) and the plots run along the direction DC, forming with AB an angle of $90° - \alpha$. Representing the length of the plots by l, their width by w, and taking as

origin of fertility the level DT, whose distance from zero is μ, the productivity of any plot can easily be determined by multiplying the area $l \times w$ by the distance of its centre of gravity from the origin $DT + \mu$. This product will have, of course, to be further multiplied by the rate of fertility γ. In the case of Plot I the productivity is given by:

$$\gamma lw \left(\mu + DP_1\right) = \gamma lw \left(\mu + DL_1 \cos \alpha\right) = \gamma lw \left(\mu + \frac{l \cos \alpha}{2} - \frac{w \sin \alpha}{2}\right).$$

In the same way the productivity of the second plot is:

$$\gamma lw \left(\mu + \frac{l \cos \alpha}{2} - \frac{3w \sin \alpha}{2}\right),$$

and that of the nth plot:

$$\gamma lw \left(\mu + \frac{l \cos \alpha}{2} - \frac{(2n - 1)\, w \sin \alpha}{2}\right).$$

Then the mean productivity of say n consecutive plots is worked out. Since the coefficients in the second part of each of the above formulae run in an arithmetical progression $[1, 3, 5 \ldots (2n-1)]$,

$$\text{Mean} = \gamma lw \left(\mu + \frac{l \cos \alpha}{2} - \frac{nw \sin \alpha}{2}\right) \qquad \ldots\ldots(1).$$

Now proceeding to the determination of the S.D. of productivity, the deviations of the individual plots from their mean (1) are first taken. The part $\gamma lw \dfrac{w \sin \alpha}{2}$ is common to all of them, while their coefficients run as follows:

$$(n - 1), (n - 3), \ldots (n - n + 2), - (n - n + 2), \ldots - (n - 1).^*$$

Squaring and summing one gets

$$\overset{n}{\underset{1}{S}} = 2\left[(n - 1)^2 + (n - 3)^2 + \ldots + 4^2 + 2^2\right]\left(\gamma lw \frac{w \sin \alpha}{2}\right)^2$$

$$= 2^3 \left[1^2 + 2^2 + \ldots + \left(\frac{n - 1}{2}\right)^2\right]\left(\gamma lw \frac{w \sin \alpha}{2}\right)^2.$$

And since $\quad 1^2 + 2^2 + 3^2 + \ldots n^2 = \dfrac{n\,(2n + 1)\,(n + 1)}{6},$

$$\overset{n}{\underset{1}{S}} = \frac{n\,(n + 1)\,(n - 1)}{3} \times \left(\gamma lw \frac{w \sin \alpha}{2}\right)^2.$$

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* These coefficients are correct only if n is an odd number. If it is an even one, then $(n - 1), (n - 3), \ldots 3, 1, - 1, - 3, \ldots - (n - 1)$ should be written instead, although the sum of their squares is given by the same formula in both cases.

Hence, dividing by $n-1$, the number of degrees of freedom, and taking the square root,

$$\text{S.D.} = \sqrt{\frac{n(n+1)}{3}} \times \gamma l w \frac{w \sin \alpha}{2} \qquad \ldots\ldots(2).$$

On the other hand, from the S.D. (2) and the mean (1) c.v. is worked out without difficulty:

$$\text{C.V.} = 100 \sqrt{\frac{n(n+1)}{3}} \times \frac{w \sin \alpha}{2\mu + l \cos \alpha - n w \sin \alpha} \qquad \ldots\ldots(3).$$

There is no doubt that the smaller the value of the c.v., the less will be the effect of soil heterogeneity. Consequently it is of considerable importance to examine which is the most satisfactory way of keeping its value as low as possible, and how the different factors n, w, l, μ and angle α affect the above formula (3).

Considering first the factors w and l, which, for a given size, determine the shape of the plots, from the above formula it is clearly shown that by decreasing the width of the plots w and correspondingly increasing their length l, the productivity of the individual plots becomes more uniform. In other words, the value of the c.v. depends directly on the value w/l, this being the most conclusive theoretical evidence of the importance of the shape of the plots in field experimentation. The ratio w/l can, of course, take any value one would think necessary, although for practical purposes it is hardly convenient to adopt a w/l less than 1/100 or at least 1/200.

With regard to the factor n, it shows the number of different treatments or varieties it is allowed to test at the same time if a certain degree of accuracy is desired. If w/l and angle α are very small, there is no real restriction concerning the number of treatments, since in any case the value of the formula (3) will be very small. If, however, this is not the case, it will be necessary to include in the same experiment only a few different varieties or treatments, because under such conditions one should not expect the first and, say, the fiftieth plot to be very much alike.

The number μ, which, if γ remains constant, is an index of fertility on the line of origin DT, suggests that, other things being equal (γ included), the value of the c.v. becomes smaller when the productivity of the field is higher. If μ is very small, the c.v. depends entirely on the other factors of formula (3); if, however, it is large, then it helps in reducing the variability of the plots. In practice this factor, apart from being absolutely out of control, does not seem to be of great significance,

because, considering that the productivity of one field (depending on μ and on γ as well) cannot usually exceed that of another more than twice or three times, the values which μ can take are very limited. This view is further supported by the fact that no matter how fertile the experimental field, the individual plots vary greatly with regard to their productivity.

Finally, angle α appears by far the most important factor affecting the value of formula (3). An increase of the angle α, from $0°$ to $90°$, implies a great increase of the c.v., because it not only makes the numerator larger, but at the same time reduces the denominator (by increasing the quantity $nw \sin \alpha$ and decreasing $l \cos \alpha$). Unfortunately angle α is almost entirely beyond control, because it is scarcely possible to determine its value even within wide limits of approximation. When angle α approaches $0°$ or $90°$, c.v. is bound to be small in the first case and large in the second, with almost any ordinary value given to the other factors contained in formula (3). Moreover, for values of angle α between these two extremes, which, as it will be seen below, are more likely to be met with under natural circumstances, c.v. depends chiefly on the ratio w/l. There is no doubt that in this respect the number n also is rather important; but apart from the fact that in practice it cannot vary so much as the fraction w/l, it should be remembered that even with the smallest possible n (equal to $3-5$), it is essential to adopt a small w/l in order to make sure of a low c.v. of the productivity of the plots.

The preceding conclusions are based on the assumption that soil fertility varies evenly and along a straight line, which obviously is not correct. The field conditions which are likely to be met with under natural circumstances are much more complicated than that, and it is necessary to enquire whether the advantage of the long plots still persists in such cases.

Suppose, for instance, that soil fertility increases irregularly along the field (at DB, EH, OP, KL, ..., Fig. 3), forming different angles with the direction of the plots ST. When Plots I and II are considered in sections (1, 2, 3 ...) I_1 is less fertile than II_1 (because the distance a_1 is smaller than b_1). However, I_2 is more fertile than II_2, again I_3 is less than II_3 and so on. Furthermore, considering the rectangles like $AFQG$, in the first case the advantage is with Plot II, since the small rectangle c is more fertile than the rectangle d, the remaining two triangles on each side of DB being of equal fertility. In the second case (rectangle $MNJI$) the advantage is with Plot II and so on. Consequently, although there

are great differences between the respective sections of adjacent plots, their effect is not cumulative, because the differences tend to cancel out in the long run.

There is no doubt that the longer the plots, the greater the probability that a better elimination of the sectional variations of the plots will be effected. The same applies to several other cases which can be devised in this connection. However, attention should be called to the following interesting point: When soil conditions become complicated (which is the rule under natural circumstances), cases where angle α
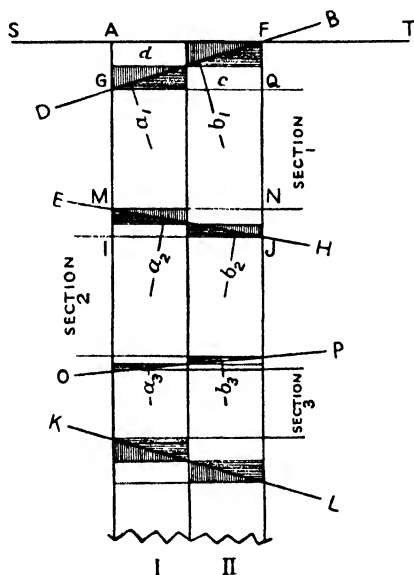
Fig. 3. Irregular change of fertility.

takes values approaching $0°$ or $90°$ are absolutely impossible, since the most favourable or unfavourable orientation of some sections of the plots are counterbalanced by opposite orientations in other sections. What should be expected is something corresponding to a value of angle α varying from, say, $30°$ to $60°$, where the effect of the shape of the plots is of considerable importance.

Concluding the above theoretical considerations with regard to the importance of the shape of the plots in field experimentation, it seems that when dealing with either patched or gradual soil heterogeneity: (1) In no case can square plots be more uniform than long ones. (2) The smaller the value of the fraction w/l, or the more complicated the change

in soil fertility, the greater the advantage of the long plots. (3) In some very rare instances their efficiency in securing uniformity in experimental conditions may be small. In such cases, a change of 90° in the orientation of the length of the plots should result in a great advantage in favour of the long plots. (4) Where there is a pronounced gradual change of the productivity of the field, it is advisable to reduce the number of the different treatments or varieties tested at the same time unless an exceptionally small fraction w/l can be adopted.

<div align="center">

EXPERIMENTAL EVIDENCE IN FAVOUR OF THE ABOVE
THEORETICAL CONCLUSIONS.

</div>

In questions like soil fertility, which depend on many complicated factors, little known and almost entirely beyond control under field conditions, one must not expect to arrive at any practical result by investigating merely the theoretical side of them. Very often the best-founded and the most convincing argumentation is valueless if a factor unsuspected, but by no means insignificant, has been overlooked. Therefore it is necessary to test whether actual experimental data agree with the foregoing theoretical considerations, because that constitutes the final and only safe proof of their validity.

Accordingly, the results of some of the best-known uniformity trials have been considered, and, although many of them are not particularly good material for testing the comparative value of different shapes of plots (w/l of the plots can scarcely be less than 1/20, which is not always sufficient to check the value of the c.v.; moreover, some systematic sources of error, like the effect of the furrow, inequality of the distances between the rows of the drill, etc., have not been carefully eliminated), it will be seen below that the results given do not lie far away from expectation.

(a) *Mercer's and Hall's experiments* (10). In this instance two fields, 1 acre each, sown with mangolds and wheat respectively, were divided up at harvest into 200 plots in the case of mangolds and 500 in the case of wheat, each of them separately harvested, thrashed and weighed. By combining these ultimate plots in different ways, it has been possible to study the variability of plots of different shapes.

It should be mentioned that, when comparing plots of different shapes, the whole experimental area was always considered divided up into the same number of plots (conditions which have been disregarded by many previous workers). And since the mean for groups of plots of different shapes was the same in each case, instead of the c.v., the s.d. has been

used as index of soil heterogeneity. Moreover, when comparing two S.D.'s the significance of their difference is determined by the z test (Fisher[3], chapter VII), and if the value of z for required n_1 and n_2 is not given in the tables, the formula

$$\text{S.D.} z = \sqrt{\frac{1}{2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

has been applied, or z was determined by interpolation.

In the case of mangolds there is no definite advantage with long narrow plots. Table I shows the results obtained when 10 ultimate plots were grouped together, forming 20 large ones (1/20 acre each) with a mean yield of 3285·8 lbs.

Table I. *Mercer's and Hall's data with mangolds. (The length of the plots runs along the direction of the rows.)*

Shape of plots

w/l	Way in which the plots have been formed		S.D.	$\log_e$ S.D.
1/1·7	Width = 5 rows of the table or	36 ft.	94·7	2·24813
	Length = 2 columns of the table or	60·50 ft.		
1/10·5	Width = 2 rows of the table or	14·40 ft.	87·7	2·17134
	Length = 5 columns of the table or	151·25 ft.		
1/42	Width = a single row of the table or	7·2 ft.	88·3	2·17816
	Length = 10 columns of the table or	302·5 ft.		

For $n_1 = 19$, $n_2 = 19$ and $P = 0.05$, z lies between 0·4182 and 0·3743, so that the above differences are not significant.

When, however, plots with their greater dimension from south to north (across the direction of the drill) were considered, an appreciable difference of the S.D. was found in favour of the longer plots, although statistically not significant.

With regard to the wheat field, Mercer and Hall did not test the effect of the shape of the plots. Later on, however, Lyon worked out the C.V. for 20 long plots (each row of the original table considered as a single plot), and 20 nearly square ones (width = 5 columns of the table, length = 5 rows). He found that, in the first case, where w/l was 1/18·6 (or 1/33·6 as the direction of the drill is not stated in the original), the C.V. V was equal to $2·3 \pm 0·36$ per cent., whereas in the second ($w/l = 1/1·33$ or 1/0·74) $V = 4·8 \pm 0·76$ per cent. The difference of $2·5 \pm 0·84$ per cent. in favour of the long plots is three times its standard error and, therefore, greatly significant.

When, however, the columns of the table were taken as single plots and compared with square ones, the effect of the shape of the plot was negligible.

It is remarkable that, in the case of mangolds, where the use of any size of plot is immaterial (Table I), the length of the plots runs along the direction of the drills. And since (p. 108) "in measuring the plot a fixed number of rows of the drill were taken..., so that if there were any variation in the breadth of the drills, the assumed acreage would not represent the actual land area of each plot," it is justifiable to hold that the inconsistency of the results in this case is due, partly at least, to the fact that the width of the columns might not have been equal, although including the same number of rows. In the case of wheat the direction of the drill is not stated, but if it is from south to north, then the inefficiency of the long plots, where their length runs parallel to the drill (along the columns of the table), can be easily explained.

In this connection one can suggest as additional causes, disturbing the normal course of events, the possibility of differences amongst the coulters of the drill and the overlapping of the end rows. Furthermore, tho wheat field was partly contaminated by thistles, which, it was found, affected the results significantly. Finally, from diagrams given in Mercer's and Hall's paper, which show the productivity of the soil for the two cases where the shape of the plots was indifferent, it is obvious that there is a gradual change of fertility from south to north in the mangold field and from west to east in that of wheat, which apparently could not be avoided with the values of w/l available, and which may be greatly responsible for the inconsistency of the results obtained.

Consequently, in view of these considerations concerning Mercer's and Hall's data, it is not surprising why long plots have not in all cases been proved more uniform than square ones.

(b) *Lyon.* Another set of experimental data used in this instance are those secured by E. G. McClosky with potatoes and mentioned by Lyon[9]. The 204 ultimate plots of the experiment were combined in three different ways, forming 34 plots in each case, 28·9 square yards each in area, with a mean yield of 137 lb. The results are contained in Table II.

Here, with a w/l equal to only $1/25·61$, it was possible to reduce the S.D. 50 per cent. of that corresponding to plots whose $w/l = 1/2·85$. The length of the plots runs across the direction of the rows and, owing to the arrangement of the experiment, it is impossible to form comparable plots with their greater dimension running along the rows.

(c) *Pearson.* Furthermore, some unpublished data by E. D. Pearson[12], secured while he was working at Cambridge under Prof. Engledow, provided another means for testing the importance of long

narrow plots. In this case 16 rows 12 ft. long and 8 in. apart were selected in 1929, each row divided into 24 equal parts 6 in. long, so that there were in all 384 small plots, for each of which the number of shoots per plot was counted on May 2 and 3. By taking together 24 adjoining plots in three different ways it has been possible to compare three shapes of plot, the number of large plots being 16 in each case, their size 1152 sq. in., and the mean number of shoots 1132 (Table III).

Table II. *McClosky's data with potatoes mentioned by Lyon.*

w/l	Way in which the plots are formed		S.D.	$\log_e$ S.D.
(1) 1/2·85	Width =3 rows of the table or 102 in. Length =2 columns of the table or 290·3 in.		19·2	0·65232
(2) 1/6·4	Width =2 rows of the table or 68 in. Length =3 columns of the table or 436 in.		17·8	0·54232
(3) 1/25·6	Width =1 row of the table or 34 in. Length =6 columns of the table or 871 in.		9·6	− 0·04082

For $n_1=33$ and $n_2=33$, S.D. $z = \sqrt{\frac{1}{2}\left(\frac{1}{33}+\frac{1}{33}\right)} = \pm0.1741$, $z_{1,2}=0.11000\pm0.1741$ (non-significant), $z_{1,3}=0.69314\pm0.1741$, greatly significant, and $z_{2,3}=0.58314\pm0.1741$, again greatly significant.

Table III. *E. D. Pearson's unpublished data (length of the plots along the drills).*

w/l	Way in which the plots are formed		S.D.	$\log_e$ S.D.
(1) 1/1·1	Width =4 columns of the table or 32 in. Length =6 rows of the table or 36 in.		101·3	2·31539
(2) 1/4·5	Width =2 columns of the table or 16 in. Length =12 rows of the table or 72 in.		60·3	1·79675
(3) 1/18	Width =1 column of the table or 8 in. Length =24 rows of the table or 144 in.		32·9	1·19089

For $n_1=15$, $n_2=15$ and $P=0.01$, $z=0.6297$. For $n_1=15$, $n_2=15$ and $P=0.05$, $z=0.4385$. In this case $z_{1,3}=0.51864$, $z_{1,3}=1.12450$ and $z_{2,3}=0.60586$, therefore all the differences are significant.

The above figures show that the effect of the shape of the plots is very great. By merely changing the value of the ratio w/l and keeping everything else absolutely the same, it has been possible to decrease the S.D. more than three times, or from 109·3 to 32·9! Moreover, it must be noticed that here the length of the plots runs parallel to the direction of the drill. If the plots are formed across the drills, the importance of their shape becomes much smaller, since for

$$w/l = 1/1.33, \text{ S.D.} = 75.1,$$

and $$w/l = 1/21.33, \text{ S.D.} = 62.2$$

($z = 0.18847$ only, while it should be 0·3425 for $P = 0.05$).

This, of course, is not unexpected after what has been said in the preceding paragraphs regarding the theoretical aspect of the problem. When the length of the plots ran along the rows of the drill, the effect of the shape of the plots was shown to be remarkable, consequently angle α must have been very small. Now changing the orientation by 90°, the corresponding angle between the direction of the plots and that of soil fertility will be approaching 90°, hence the decreased effectiveness of the shape of plot.

(d) *Kiesselbach.* Kiesselbach's[6] data with oats bring further evidence in favour of the long plot. The original experiment consisted of 207 1/30th acre plots arranged in three columns. Taking three plots at the same time in two different ways, it has been possible to get 69 1/10th acre plots in each case (mean yield = 234·3 lb.), with the result that the longer plots were found to be more uniform than square ones. The figures actually got are shown in Table IV.

Table IV.

w/l	Way in which the plots are formed		S.D.	$\log_e$ S.D.
1/16	Width = 3 rows of the table or	198 in. }	14·5	0·37156
	Length = 1 column of the table or	88 yd. }		
1/144	Width = 1 row of the table or	66 in. }	10·1	0·00995
	Length = 3 columns of the table or 264 yd. }			

$$z = 0·36161 \pm 0·12127.$$

Here again the difference is statistically significant and in favour of the long narrow plots.

(e) *Stephens.* Stephens'[14] uniformity trials are also worth mentioning. He harvested and weighed separately the yield (green forage) of not less than 2000 small sorghum plots, about 1/800th acre each. Discarding the two outside rows on each side of the field and the first and last rods for border effect, there remain 1728 ultimate plots, which combined in different ways provide groups of various shapes.

When the length of the plots runs across the rows of the field, 18 plots about 1/9th acre each were formed in three different ways (mean yield = 17,271·7 oz.), giving the results shown in Table V.

Table V.

w/l	Way in which the plots are formed		S.D.	$\log_e$ S.D.
(1) 1/1·21	Width = 4 columns of the table or	22 yd. }	975	2·27727
	Length = 24 rows of the table or	80 ft. }		
(2) 1/4·84	Width = 2 columns of the table or	11 yd. }	712	1·96291
	Length = 48 rows of the table or	160 ft. }		
(3) 1/19·4	Width = 1 column of the table or	5·5 yd. }	366	1·29746
	Length = 96 rows of the table or	320 ft. }		

$$z_{1,2} = 0·31436 \pm 0·24272. \quad z_{1,3} = 0·97981 \pm 0·24272. \quad z_{2,3} = 0·66545 \pm 0·24272.$$

This, of course, means that long plots have an unmistakable advantage over square ones.

If, however, the length of the plots runs along the rows, their shape has no effect at all. It must be noticed, though, that in this case the longest plots are simply the individual rows of the drill. Moreover, the field containing the 2000 plots was originally drilled "to obtain an increased quantity of seed, and only later in the year as harvest approached, the idea developed of using it for estimating the experimental error." Under these ordinary circumstances it is not unlikely that no special precautions were taken to secure a uniform spacing amongst the rows of the field, or, as is pointed out in the original, to avoid other sources of error apart from soil heterogeneity. Consequently the discrepancies
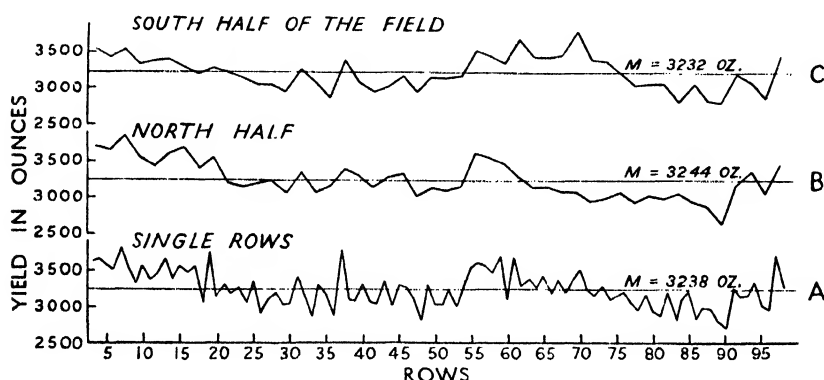
Fig. 4. Stephens' data with sorghum, A showing the yield of single rows and B and C that of two adjcent half-rows.

observed may be attributed to these causes, some evidence for which is provided by Fig. 4 (A, B, C). A, showing the yields of individual rows, presents an unusual variation amongst adjacent rows, although the general trend of fertility is fairly uniform: whereas B and C, where the yields of two consecutive half-rows have been added together, are far more regular in that respect, but with more marked changes from one part of the field to the other.

(f) *Data secured by the author at Cambridge.* In order to get additional evidence on the effect of the shape of plots, two blocks (24 rows each) were set aside this year on an ordinary field of the University Farm. At harvest every row was divided into 12 parts, so that each block consisted of 288 small plots.

In block A the rows were 90 ft. long and, since the distance between

these was 8 in. (in this respect discrepancies of $\frac{1}{2}$–1 in. were not rare), each ultimate unit was 8 in. × 7$\frac{1}{2}$ ft. in area. These small plots were harvested[1] and thrashed separately and the yield of grains weighed to the nearest gram twice with the same result. The figures obtained are given in Table VI.

Table VI. *Block A. The yield of* 288 *ultimate plots*
(*to the nearest gram*).

Rows	\multicolumn{12}{c}{Sections}	Total of rows
	1	2	3	4	5	6	7	8	9	10	11	12	
I	85	67	49	73	79	68	54	74	64	73	61	81	828
II	87	61	79	72	89	79	48	78	63	60	59	82	857
III	88	78	70	92	101	71	50	95	69	76	64	75	929
IV	100	81	76	79	85	77	45	88	71	82	59	86	929
V	76	72	86	78	100	62	52	80	78	87	68	79	918
VI	76	82	77	70	88	74	51	85	58	65	56	74	856
VII	78	86	68	77	86	83	54	88	61	72	72	84	909
VIII	94	84	71	79	90	75	44	67	65	76	65	70	880
IX	79	67	60	63	77	72	43	68	72	70	54	74	799
X	74	84	72	66	78	70	55	66	76	81	52	57	831
XI	92	66	65	52	58	73	51	50	36	81	42	78	744
XII	61	65	50	51	86	83	87	75	95	118	60	88	919
Total of half-columns	990	893	823	852	1017	887	634	914	808	941	712	928	10,399
XIII	76	67	60	72	91	79	65	63	91	102	77	97	940
XIV	63	59	60	67	69	85	48	51	69	80	58	87	796
XV	56	65	57	59	72	69	44	46	90	96	54	84	792
XVI	79	61	57	63	70	80	52	45	82	105	58	74	826
XVII	82	58	64	65	77	62	44	39	68	62	39	65	725
XVIII	62	63	60	45	62	77	54	48	55	55	54	55	690
XIX	63	75	56	77	96	65	60	58	73	72	53	88	836
XX	70	81	76	84	66	64	59	62	72	75	46	80	835
XXI	85	75	77	67	83	78	69	57	75	97	55	77	895
XXII	90	80	66	81	67	81	65	52	67	84	58	77	868
XXIII	83	89	74	79	87	71	56	52	64	91	67	74	887
XXIV	85	69	63	62	82	94	53	37	55	87	61	69	817
Total of half-columns	894	842	770	821	922	905	669	610	861	1006	680	927	9907

Grand total   20,306

By combining 12 of the ultimate plots in various ways, six different shapes were compared (for each shape 24 large plots were available, 5 sq. yards in area, their mean yield being 846·1 gm.) with the result shown in Table VII.

In block *B* the rows were 102 ft. long, and the size of the ultimate plots 8 in. × 8 ft. In order to save space the yields of the individual plots are not given. When, however, the figures were treated as those of block *A*, the result was as shown in Table VIII.

[1] The author is indebted to Dr Sanders, Mr Garner and their class of post-graduates for the great help they gave him at harvest.

Table VII.

w/l	Way in which the plots are formed	s.d. (% of the mean)	$\log_e$ s.d.
1/135	Single rows	7·86	2·06179
1/33·75	Length = 6 columns of the table or 45 ft. Width = 2 rows of the table or　16 in.	9·23	2·22354
1/15	Length = 4 columns of the table or 30 ft. Width = 3 rows of the table or　2 ft.	7·71	2·04252
1/8·44	Length = 3 columns of the table or 22½ ft. Width = 4 rows of the table or　21·8 in.	10·01	2·30353
1/3·75	Length = 2 columns of the table or 15 ft. Width = 6 rows of the table or　4 ft.	11·37	2·43089
1/1·06	Length = 1 column of the table or　7½ ft. Width = 12 rows of the table or　8　ft.	13·82	2·62614

Table VIII.

w/l	s.d. (% of the mean)	$\log_e$ s.d.
1/153	7·96	2·07443
1/38·25	8·43	2·13180
1/17·40	8·96	2·19277
1/9·56	10·66	2·36657
1/4·25	9·92	2·29455
1/1·06	10·48	2·34942

Since, for $n_1 = 23$, $n_2 = 23$, and $P = 5$ per cent., $z = 0\cdot3502$, it is obvious that some differences in block A are statistically significant even when considered singly. If, however, it be taken into account that in both cases the s.d. constantly decreases with a decreasing value w/l, the result as a whole is very conclusive[1].

The experimental data discussed in the preceding paragraphs furnish strong evidence in favour of the assumptions made with regard to the shape of plots. In no case were square plots found more uniform than long and narrow ones. On the contrary, in the majority of cases the advantage of the long plots was manifest, while three of them (two provided by Mercer's and Hall's data and the third by Stephens') did not show any indication in favour of either shape. It must not be forgotten, however, that the last three inconsistent cases are open to criticism owing to the evidence brought forth that apart from soil heterogeneity other experimental conditions also have not been kept properly under control.

[1] Dr Fisher has kindly pointed out that when comparing the first standard deviation with the second, the second with the third, etc., the z test is too severe, owing to a certain correlation existing between the respective plots. With a more refined method the significance would be greater than that shown.

PRACTICAL APPLICATIONS.

In the light of the foregoing results concerning the effect of the shape of plots, one naturally would always recommend the use of long narrow plots when carrying out experiments under field conditions. In practice, however, before long plots are definitely adopted, there are some other questions to be carefully considered, which will be dealt with in the following paragraphs.

(*a*) *Competition.* It has been shown that when two varieties, differing with regard to their habits of growth, etc. are grown side by side, the adjacent rows interfere with each other in development and yielding capacity. This effect, usually known as competition, disturbs the normal course of events, and presumably constitutes another factor responsible for the variability of the results in yield trials. There is no doubt that the larger the proportion of the adjacent rows to the whole area of the plot, the greater the effect of competition. And since by using long narrow plots an increase of this proportion is unavoidable, it is obvious that the question of competition is closely connected with that of the shape of plots.

Experiments on competition were probably carried out for the first time by Montgomery (11), who showed that individual plants are greatly affected by their neighbours, and that the comparative yielding values of two varieties grown alone were often reversed when planted in competition. Hayes and Arny (5), however, testing the effect of competition by different correlation coefficients, did not come to any definite conclusion, since out of eight cases considered only one showed a really significant result. Kiesselbach (6), on the other hand, who supplied considerable experimental data on this subject, pointed out that in field experimentation competition is an important factor which must be taken seriously into account. His work, however, has been criticised rigorously by Love (8), who, denying the effect of competition, states that between the ordinary varieties usually tested in any one locality there is very little competition. Later on Stadler (13), working on the same lines as Kiesselbach, found that the effect of competition is by no means negligible, in some cases changing the yielding ability of different varieties grown side by side by as much as 100 per cent. Moreover, in 1923 Kiesselbach (7) published the results of further experiments with maize, which gave additional evidence of the "unequal competition between adjacent hills or rows, containing unlike sorts or unlike stands of corn." Finally, more recent work carried out by Stringfield (15) is of great interest

in this respect. Applying different methods for detecting any competitive effect and using a large number of replications in each case, he was led to the following conclusion: "Competition is undoubtedly playing some part in modifying yields, but its influence seems to be so weak that grosser errors obscure it....If varieties generally similar in growth and time of maturity are grouped, there seems to be little reason for further concern about the matter of competition."

In spite of the great divergence of opinion which exists amongst the above-mentioned investigators regarding the effect of competition, and which is bound to continue as long as different plants and different varieties are tested under different environmental circumstances, there are two or three points on which all of them seem to agree, and which are of great importance for our purpose. It is almost universally recognised that: (1) competition does exist at least in some cases, (2) its effect (if any) is limited to only one row on each side of the plot, and (3) when varieties similar in their habits of growth and maturity are grouped together the effect of competition is negligible.

Therefore, when using long and narrow experimental plots in order to avoid any undesirable competitive effect it will only be necessary to choose with discrimination the varieties which are going to be tested together. And since it is sometimes necessary to include in the same group varieties (or any kind of treatment) suspected to disturb the normal development of their neighbours, in such cases the narrowest plot permissible is a three-row one, from which the two outside rows are discarded. Unless, however, one is fairly certain about the border effect, it is advisable to harvest the three rows separately and discard the edge ones only if competition is really significant, otherwise taking all of them into account. In this way much experience can gradually be accumulated regarding competition under definite circumstances.

(b) *Convenience.* Although a very small w/l (say 1/100) is desirable on theoretical grounds in yield trials, it may often be necessary to adopt a comparatively large w/l on account of some other essential practical considerations. For instance, in small scale experiments the use of a very long and narrow plot may sometimes involve either single-row plots, which should be avoided in order to remain on the safe side with regard to competition, or a great amount of seed not available in certain cases. Likewise, in farm-scale experimentation the length of the plot is determined by the length of the field, while the breadth depends on the width of the drills and other machinery used, so that w/l cannot be fixed quite at will. On the other hand, with some crops like cotton, tobacco, sugar-

beet, etc., planted in rows 2 or 3 ft. apart, it is doubtful if it will be wise to adopt a plot 200 or 300 yds. long, in order to keep w/l equal to or less than 1/100 (using three-row plots). The above and other similar practical difficulties included under the general term convenience, show that in practice the value w/l could not be less than a certain limiting value. On the contrary, owing to the lack of practical experience regarding the use of long narrow plots, it seems advisable that before actually adopting them one should pay due attention to every little point of detail.

(c) *Factors affecting yield and soil fertility in a definite direction.* It should be noted that, with regard to uniformity, long plots suffer more than square ones from factors altering soil productivity in a definite direction, when these factors work in a direction parallel to the length of the plots. Such instances are not uncommon in practice, considering that many of the operations of the field are executed chiefly by implements along a straight line, and that very often the plots are arranged along the same direction. Therefore, with long plots great care is required in order to avoid a critical value for angle α (sometimes liable to be exactly 90°), which would make ineffective even the smallest possible w/l.

This applies primarily to the width of the plots. Since we want to keep this as small as possible, and in ordinary cases it will rarely be larger than 1 yard, it is essential to measure it with the greatest accuracy, because a discrepancy of 2 inches, or even 1, will affect the results significantly. Fortunately this is not a very difficult achievement, because in cases where sowing is done by the dibbing iron, the standard of accuracy is usually very high; on the other hand, if small three- or five-row drills are used, they are operated by workmen, and everything depends on their skill and conscientiousness as in any other case. At any rate the measuring of the width of the plots is probably the weakest point in adopting a very small w/l, and in view of the observations previously made in connection with some experiments suspected as not quite satisfactory in this respect, too much stress cannot be laid on this point. The same, of course, applies to the distance between the rows and the uniformity of the coulters of the drill, where even the slightest inaccuracy is not permissible.

Moreover, former paths, drainage pipes, the furrow left after ploughing, and other factors of this kind, may greatly affect the fertility of the field, and if coinciding with the length of the plots, will alter materially the uniformity of the long plots. In such instances precautions should be

taken that angle α does not reach a value approaching $90°$, and consequently a suitable orientation of the plots should be chosen.

(*d*) *The arrangement of the experiment and the determination of the standard error.* Long and narrow plots do not lend themselves to a Latin Square arrangement, which at the present time is the most favoured by investigators anxious to frame their experiments in accordance with the requirements of modern statistical technique. However, they are particularly suitable for the "randomised blocks" system, and there is no doubt that with long plots this is by far the most satisfactory method for laying-out an experiment.

With regard to the procedure which should be followed in determining the experimental error the "Analysis of Variance" (Fisher[3], Fisher and Wishart[4]) appears to be without competitors. Nevertheless, it should be remembered that the "Analysis of Variance" differs only in appearance from "Student's" method[16] and in so far as the randomised block arrangement is concerned it is absolutely the same. "Student's" formula

$$\sigma_e{}^2 = \frac{mn\left(\sigma_T{}^2 - \sigma_R{}^2 - \sigma_G{}^2\right)}{(m-1)(n-1)},$$

for the final form of which credit is given to R. A. Fisher, shows exactly what one has to do when applying the analysis of variance. In order to make it clearer it is only necessary to rewrite the above formula under Fisher's notation

$$\sigma_e{}^2 = \frac{\overset{mn}{\underset{1}{S}}(x_T - \bar{x}_T)^2 - n\overset{m}{\underset{1}{S}}(\bar{x}_R - \bar{x}_T)^2 - m\overset{n}{\underset{1}{S}}(\bar{x}_G - \bar{x}_T)^2}{(m-1)(n-1)},$$

when it can be easily seen that the numerator represents the sum of squares left for the determination of the error, and the denominator the number of the corresponding degrees of freedom.

In the case of the modification introduced by Engledow and Yule[2], the formula

$$\sigma_d{}^2 = \frac{2m}{m-1}\left(\sigma_v{}^2 - \sigma_p{}^2\right),$$

which they arrived at by a very simple algebraical process, is similar to that given by "Student," the only difference being that when calculating the variance, they divide by n instead of $n-1$, the number of degrees of freedom. Moreover, they have already multiplied by 2 to get the variance of a difference. The routine of the arithmetic is here entirely

different, because $\sigma_v{}^2$ is calculated instead of $\sigma_T{}^2 - \sigma_G{}^2$. Nevertheless, making allowance for the two points above mentioned, one should always get the same result by either Engledow's and Yule's formula or by the analysis of variance.

In this connection another question has to be considered. The S.D. of the uniformity trials previously dealt with has been obtained by dividing the total sum of squares by the total number of degrees of freedom. In an ordinary experiment, where the analysis of variance is applied properly, these totals are decreased by the values corresponding to grouping, treatments, etc., so that the sum of squares and the number of degrees of freedom due only to error is taken into account. Consequently it might be asked whether the advantage of the long plots holds good in the case of uniformity trials treated as randomised blocks (advocated for long plots), where an allowance for grouping is duly made.

Supposing that S_e and S_g are the sums of squares due to error and grouping, and F_e and F_g the numbers of their respective degrees of freedom; then $\dfrac{Se + Sg}{Fe + Fg}$ and $\dfrac{Se}{Fe}$ represent the variance of the error without or with allowance for grouping. It is obvious that the process of grouping will result in a smaller estimate of the variance if only

$$\frac{Se}{Fe} < \frac{Se + Sg}{Fe + Fg} \quad \text{or} \quad \frac{Se}{Fe} < \frac{Sg}{Fg}$$

(*i.e.* if the variance of the error is smaller than that of the groups). In cases where $S_e/F_e = S_g/F_g$ grouping will be without effect, and if $S_e/F_e > S_g/F_g$ it will give a larger variance. In other words, if the variation between blocks is not sufficiently *larger* than that between individual plots, grouping should be expected to give a *higher* value for the variance, and *vice-versa*.

Furthermore, according to the experimental results discussed in earlier paragraphs of this paper, long plots are comparatively uniform and highly correlated with each other, whereas square ones present a much greater variability. On the other hand, groups of long plots are much more variable in comparison with the ultimate long plots than groups of square plots respectively, because in the first case grouping involves an increased distance between the centres of gravity of the blocks and an increase of w/l proportional to the *number* of plots contained in each block. In the second, however, the increase in distance is only proportional to the *square root of that number* if w/l of the blocks is strictly kept

equal to unity, becoming still smaller when w/l takes values less than unity (which is more usual).

Therefore, when applying the analysis of variance in experiments where long narrow plots have been used, the sum of squares due to grouping, being comparatively large, will reduce the total sum of squares appreciably, resulting in a smaller estimate of the error. On the contrary, if the preference was given to square plots, the variation of the blocks will be rather small and, after the correction for grouping, the experimental error may even increase instead of becoming smaller. This means that when the analysis of variance is applied properly, the difference in accuracy between long and square plots is accentuated in favour of the former rather than otherwise.

It might be interesting to mention that in two instances where experimental data have been considered from this point of view, the results showed an extremely close agreement with expectation. From Pearson's data it was found (p. 25) that:

For $w/l = 1/1\cdot1$,

<div style="text-align:center">S.D. = 101·3 or 8·95 per cent. of the mean.</div>

For $w/l = 1/18$,

<div style="text-align:center">S.D. = 32·9 or 2·9   ,,   ,,</div>

When the 16 plots were grouped in four blocks and their sum of squares properly subtracted from the total, then:

For $w/l = 1/1\cdot1$,

$$\text{S.D.} = \begin{cases} 111\cdot1 \text{ or } 9\cdot8 & \text{per cent. of the mean.} \\ 108\cdot3 \text{ or } 9\cdot57 & ,, \qquad\qquad ,, \\ 108\cdot5 \text{ or } 9\cdot57 & ,, \qquad\qquad ,, \end{cases}$$

(In this case blocks of four plots can be formed in three different ways.)

For $w/l = 1/18$,

<div style="text-align:center">S.D. = 29·1 or 2·56 per cent. of the mean.</div>

Again, with Stephens' data (p. 26) the results were as follows with ungrouped plots:

For $w/l = 1/1\cdot21$,

<div style="text-align:center">S.D. = 975 or 5·56 per cent. of the mean.</div>

For $w/l = 1/19\cdot4$,

<div style="text-align:center">S.D. = 366 or 2·12   ,,   ,,</div>

When the 18 plots were grouped in three blocks, then:

For $w/l = 1/1·21$,

         s.d. $= 1030$ or $5·96$ per cent. of the mean.

For $w/l = 1/19·4$,

         s.d. $= 345$ or $1·99$,,   ,,

While the differences are not significant it may well be that, in both cases, grouping has increased the advantage of the long plots, not only making their s.d. smaller, but at the same time resulting in a higher value for the s.d. of the square plots.

Conclusions.

1. In agricultural experiments it seems that significant results cannot be secured by only using appropriate statistical methods; uniformity amongst the individual plots is more essential than anything else.

2. Some theoretical considerations suggest that the shape of the plots constitutes an important means of controlling soil heterogeneity. In accordance with these: (*a*) in no case can square plots be more uniform than long and narrow ones; (*b*) the smaller the value w/l the more uniform the experimental plots, and (*c*) since uniformity depends (apart from w/l) on the value of the angle a, in some exceptional cases (soil fertility varying gradually and evenly, and angle a approaching $90°$) the advantage of the long plots may be less than would be anticipated. This, however, is most unlikely on account of the complexity of the variation in soil conditions and the possibility of easily avoiding such a critical value of the angle a.

3. In order to test the validity of the assumptions made regarding the effect of the shape of the plots, the numerical data of several uniformity trials have been considered. A close agreement was found between expectation and actual results, in the great majority of cases the evidence being remarkably significant in favour of the long plots. In only three cases were the results inconclusive, this apparently being accounted for by the way in which the original plots were formed, causing an inequality in area amongst them.

4. In the light of these investigations, in order to reduce the effect of soil heterogeneity, the plots used should be as long and narrow as possible. This, of course, within the limits set by different practical considerations, amongst which convenience, competition (when acting), and the accurate measurement of the width appear to be the most important.

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THE MICROBIOLOGY OF FARMYARD MANURE DECOMPOSITION IN SOIL.

I. CHANGES IN THE MICROFLORA, AND THEIR RELATION TO NITRIFICATION.

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(With Thirteen Text-figures.)

INTRODUCTORY.

IT is a well-known fact that farmyard manure, the oldest and most widely used nitrogenous fertiliser, shows a fertilising effect which is much smaller than should correspond to its total content of N, if this were fully available to the plants. The continuous wheat plots on Broadbalk, Rothamsted, the oldest field experiments dealing with this subject, have shown a utilisation by the wheat plants of 26 per cent. of the total amount of N added as farmyard manure over the period 1865 to 1912 (Russell(66)). Other investigators also give figures which, though variable, show incomplete utilisation of the manure N, as shown below.

Character of experiment	% of manure N utilised	Author
4 years field experiments	7–46	Schultze*
3 „ „	32–51	Welbel*
2 „ „	28–34	Schneidewind*
3 „ pot experiments	31–41 ⎫	Pfeiffer(56)
3 „ field experiments	92–93 ⎭	
2 „ pot experiments	0–40	Löhnis and Smith(42)
10 „ field experiments	15	Lipman and Blair(39)
1 year pot experiments	3–31	Goetors(21)
1 „ „	13–24	Glathe(20)
1 „ „	4–18 ⎫	Gerlach and Seidel(19)
1 „ field experiments	8–17 ⎭	
2 years cylinder experiments	About 30	Balks(4) and Bach(3)
8 „ field experiments	28–68	Iversen(29)

* Cit. after Löhnis(40).

This incomplete uptake of manure N by the plant is associated with its incomplete nitrification in soil. Tuxen (75) found only 33 per cent. of the N of farmyard manure nitrified in loam soil after 5 months, and after 15 months there was no appreciable change; in a sandy soil only 10 per

cent. was nitrified after 10 months, but 56–74 per cent. after 13–15 months. In the same soils bone meal and guano were nitrified to an extent of 40–72 per cent. after 1–3 months. Dehérain[13] found 15-27 per cent. of the N of various samples of farmyard manure leached out of unplanted soil as nitrate during one year. Wagner[77] found that in a 522 days' laboratory experiment 39–40 per cent. of manure N was nitrified, whereas blood meal and lucerne showed a nitrification of 73–75 per cent. Popp[58] found 33–34 per cent. of manure N nitrified after 6 weeks, but no considerable further nitrification after 12 weeks. Löhnis and Smith[42] found that variously treated samples of manure were nitrified to an extent of 18–50 per cent. in 6–12 weeks, the nitrification being best where urine had been added to the manure. The most complete series of experiments in this direction has been carried out by Barthel and Bengtsson[5, 6], who arrived at the final conclusion that only the ammonia N of well-fermented manure will undergo any nitrification during the first 12–14 months in soils of different character with or without addition of lime. This interesting result would fully explain the slow fertilising action and incomplete nitrification of manure N. Somewhat different results, however, have been obtained by Sebelien[71], who found that 30–40 per cent. of the N of urine-free faeces of cattle and horses was nitrified in moist soil within 6 months, and by the writer[30], who found a nitrification of about 25 per cent. of the N of fresh, ammonia-free manure in the same time. Also Glathe[20], Goeters[21] and Scheibe[69] found in several instances a nitrification of more N than corresponded to the content of ammonia in the manure. The reservation should here be made that none of these experiments has been carried out with manure of quite the same kind as that used by Barthel and Bengtsson—ordinary, well-fermented and decomposed farmyard manure.

We find an analogy to the incomplete nitrification of manure N in the case of other organic N compounds, such as bone meal, guano, blood meal and lucerne as mentioned above. There is further evidence in the literature (Withers and Fraps[88], Popp[58], Wright[90], Hill[24], Lathrop[34], Honcamp[27], Martin[47], Holtz and Singleton[26], Jensen[30]) to show that nitrogenous organic compounds, when added to the soil, undergo a more or less rapid nitrification (sometimes preceded by a temporary depression of nitrate formation) which gradually slows down and tends to come to a standstill before all N has been nitrified. This gradual slowing down of the process of decomposition was observed as early as 1886 by Wollny[89], who enunciated the principle that the further a substance has been decomposed, the more slowly does the

decomposition of the residue proceed. There seem to be very few exceptions to this rule of incomplete nitrification. Whiting and Richmond [84] found that leaves of sweet clover were completely nitrified after 3 months with an apparent stimulation of the nitrification of the soil's own N after 8 months but, unfortunately, it is not clear from their data whether the nitrate formed from the soil organic matter itself has been taken into account. Löhnis [43] found, in a very interesting series of experiments, that an addition of small amounts of young plant material had a tendency to stimulate the decomposition of humus in a fertile soil. It would *a priori* be expected that a certain proportion of the organic N would remain undecomposed as microbial substance. This has been suggested by Ramann [61]: "Da die Zerstörung jedoch niemals vollständig wird, sondern mindestens die Leiber der zuletzt tätigen Organismen übrig bleiben müssen, verläuft der Vorgang asymptotisch, er nähert sich dem Nullpunkt ohne ihn doch jemals zu erreichen." The residue of N, which remains as microbial substance, is obviously greater, the more the energy material supplied in proportion to the amount of N. This accounts for the harmful influence of straw on the action of manure (Krüger and Schneidewind [33], v. May [48], Hansen [23], Niklewsky [52]), which was first ascribed to denitrification, but which has later, perhaps in a somewhat one-sided way, been explained exclusively as due to the formation of microbial protoplasm (Pfeiffer and Lemmermann [57], Murray [50], Rahn [60], Allison [1]). This leads us to the question of the importance of the C:N ratio, which was first systematically studied by Doryland [15], although attention had been called to it before by Löhnis [40], and which has recently been discussed in detail especially by Waksman and his co-workers [79, 83]. Farmyard manure is an organic material of a rather low N content (C:N–15–20:1), from which we would expect a rather slow and incomplete nitrification, such as is found in experiments. One would also expect a very large accumulation of organic N compounds in continuously manured soils, owing to the incomplete utilisation of the N by the plants. This is the case to a certain extent only. Tuxen [76] found, in soils fertilised for from 22 and 30 years, a very notable increase in N due to farmyard manure, but very little due to artificial fertilisers. The experiments on Broadbalk (Russell [66]) show that during 47 years only about 14 per cent. of the N of the added manure has been accumulated in humus; about 25 per cent. has been utilised by the plants, and the remaining 60 per cent. appears to be lost, probably as gaseous N, since there is very little drainage from the field. Similar losses of manure N have been recorded in America by Lipman and Blair [39], from whose

data it is seen that about 55 per cent. manure N has been stored in the soil and about 30 per cent. lost, and in Denmark by Christensen (9), who found in soils from fertilising experiments recorded by Iversen (29) a N storage (expressed as excess in N of farmyard manured plots over artificially fertilised plots) of 14–17 per cent. of the total amount of N supplied as farmyard manure during 8 years; 28 to 68 per cent. of the N had been utilised by the plants, so that here also there is a notable loss of N. Whether this is due to leaching is uncertain, since no analyses of drainage water were made.

The present work was designed to give some information on the following questions:

I. Is the relatively low nitrification of the organic N of farmyard manure a general rule?

II. Is a part of the N of the manure in itself inaccessible to the attack of the soil micro-organisms, or can the incomplete nitrification and utilisation of the manured N by the plants be explained through the C:N ratio of the manure, *i.e.* the synthesis of new microbial protoplasm?

III. Does a loss of manure N occur in laboratory experiments under conditions where leaching is excluded?

The following sets of experiments were carried out:

1. Various kinds of farmyard manure were allowed to decompose in soils of different character, and the development of bacteria, actino-mycetes and fungi was compared with the formation of nitrate and the disappearance of carbon. Amounts of "humic acid" and possible losses of total N were estimated.

2. The nature of the micro-organisms decomposing the cellulosic material of the manure in the soil was studied, and the organisms were tested for their nitrogen requirements and their ability to decompose lignified cellulose.

3. Various kinds of microbial protoplasm were submitted to decom-position experiments in soil and sand, and in order to ascertain firstly whether the hypothesis that bacterial matter is particularly resistant to decomposition is tenable, and secondly whether resistant, humus-like nitrogenous compounds are of common occurrence in the protoplasm of micro-organisms.

Experiments falling in the first section are described in the present paper. Later contributions will deal with the two others.

(1) *Technique.*

The decomposition experiments described below were carried out in the same manner as those of Barthel and Bengtsson: soil with addition of manure or other organic material, as well as control soil without addition, was kept in wide-mouthed glass bottles, closed with tight-fitting corks, with holes through which passed glass tubes approximately 1 cm. wide filled with cotton-wool; this arrangement admits the air fully and reduces the evaporation of moisture to a minimum. The bottles were of such a size that the diameter and the depth of the soil mass were about equal at the start of the experiment. 1·2–1·5 kg. of soil was used for the long-period experiments. Samples were taken, each time after careful mixing, at various periods of time, and estimations were made of the numbers of bacteria, actinomycetes and fungi, amounts of NO_3-N and NH_4-N, and in some cases, especially at start and end of experiments, of total N, total carbon, α-humus, and its N content. The numbers of micro-organisms were calculated on the basis of fresh, moist soil, while the soil samples for chemical determinations were dried overnight at about 35° C. and the result calculated on the basis of air-dry soil.

The following methods were used:

Total nitrogen was determined by the routine Kjeldahl method; when soils containing considerable amounts of nitrate were to be analysed, the method for including nitrate (digestion with phenoldisulphonic acid and reduction with sodium thiosulphate) was used.

Nitrate nitrogen was determined by the Devarda method.

Ammonium nitrogen was determined by Bengtsson's KCl method: repeated extraction of the soil with 0·5 per cent. KCl solution and distillation of the extract with magnesium oxide.

Total carbon was determined by the simplified combustion method of Dennstedt[14].

Humus (α-humus, crude mixture of humic and hymatomelanic acids) was determined by the method of Waksman[81]: twice repeated extraction of the soil with 2·5 per cent. NaOH solution by heating for 30 minutes in the autoclave at 15 lb. pressure; the solution is filtered off, the soil is washed with NaOH solution and water, and the humus is precipitated from the extract with HCl; after sedimentation the precipitate is filtered off on weighed filter-paper, washed and dried for 20–24 hours at 55° C.

Numbers of bacteria and actinomycetes were determined by the plate

method: 10 gm. of moist soil were shaken for 4 minutes with 250 c.c. of a solution of 0·5 per cent. NaCl and 0·05 per cent. MgSO$_4$, and from a suspension diluted to 1:250,000, five or six parallel plates were poured and incubated for 10 days at 20° C. Two different agar media were at first compared. The first was the mannite-asparagine agar generally used for counting soil bacteria in this laboratory (Thornton[73]). This medium has been found by Fisher, Thornton and Mackenzie[16] to furnish counts which are in agreement with theoretical requirements, *i.e.* the numbers of colonies appearing on the plates depend only on the numbers of bacterial cells in the inoculum. The second medium was a modification of the albumen agar recommended by Waksman[78]: dextrose 2·0 gm., casein, dissolved in 0·1 N NaOH, 0·2 gm., K$_2$HPO$_4$ 0·5 gm., MgSO$_4$ 0·2 gm., agar 15 gm., H$_2$O 1000 c.c., pH 6·5–6·6. This medium had previously been used by the writer in making counts from several Danish soils (see Christensen[9]), and since it had been found then to furnish good results, it was thought worth while to compare it with the mannite agar, the only medium for which the index of dispersion $\chi^2 = \dfrac{S(x - \bar{x})^2}{\bar{x}}$ (Fisher[17]) has yet been calculated. The results showed that the casein agar gave as good a distribution of the χ^2 values as the mannite agar (especially for the actinomycetes counts which showed subnormal variation on mannite agar), and since the casein medium gave constantly higher counts, it was afterwards used exclusively.

Counts of fungi were made by the method suggested by Brierley *et al.*[8]: soil suspension was shaken as uniformly as possible for 20 minutes, using flasks of similar shape and size in all cases. Suspension diluted to 1:1000 (or a higher dilution for soils particularly rich in fungi) was plated out on Conn's glycerin agar of pH 4·6–4·8. Six parallel plates were incubated for 7 days at 25° C.

(2) *First series of experiments.*

In this series the decomposition of manure alone and mixed with straw was studied in acid and neutral soil. Two soils were used, both from the Rothamsted Grass plots; they were heavy clay soils, rich in organic matter. The first was from unmanured plot No. 1 of pH 7·0, the second from plot No. 14A (unlimed, mineral fertiliser + (NH$_4$)$_2$SO$_4$) of pH 3·8. The air-dried soils received 30 and 33 per cent. H$_2$O, and further, on air-dry basis:

(1) No addition (control soil).
(2) Farmyard manure corresponding to 2 per cent. dry matter.
(3) Same + 2 per cent. ground oat straw.

The manure was old, well decomposed and humified, but contained, probably owing to bad storage, very little soluble nitrogen. The composition of the manure and of the straw were:

	Manure	Straw
Dry matter (%)	28·0	92·3
Total N, % of dry matter	1·96	0·52
NH$_4$-N	0·10	—
Total C	29·2	43·3
Ash	40·6	5·7
α-Humus	14·1	—
% N content of α-humus	2·57	—

The experiments were run at room temperature for 500 days, and complete analyses were made after 300 days and at the end. The counts of micro-organisms are seen in Figs. 1–2 and the chemical changes in Tables I–II.

In the neutral control soil the numbers of bacteria did not undergo any very marked changes beyond the fluctuations which these figures normally show, especially in air-dried and re-moistened soil. The marked fall in bacterial numbers in soils kept in bottles in the laboratory, which has been observed by Cutler and Dixon [10], did not appear in these counts on casein agar, but was very conspicuous in counts on mannite agar after 100–125 days. In the soil + manure there was a very great increase in the numbers of bacteria after 10 days, but the numbers fell as rapidly and slowly approached those in the control soil, so that after 150 days the figures from the two soils showed no significant differences. In the soil with manure + straw there was, as might be expected, an enormous increase in bacterial numbers. This increase lasted longer, but the general trend of the figures was the same as in the previous soil: the increase was followed by a decrease which was rapid at first, then slower, so that the numbers gradually approached those of the control soil and eventually reached them, although the influence of the straw was still quite noticeable after 200–250 days. The actinomycetes behaved similarly to the bacteria, save that the changes in their numbers were less pronounced and that their numbers tended to become relatively higher with advancing time. The fungi were not at all affected by the addition of manure, but the extra addition of straw caused a very great increase in their numbers, an increase which was probably due to vegetative growth and not merely to sporulation, since it was, like that of the bacteria, only temporary. A high count of fungi, due to resting spores, would probably remain constant for a much longer period. The increase was almost entirely due to one particular fungus (*Cephalosporium* sp.)

H. L. JENSEN

which probably utilised the sugars and pentosans of the straw, since it did not grow on filter paper cellulose in pure culture.
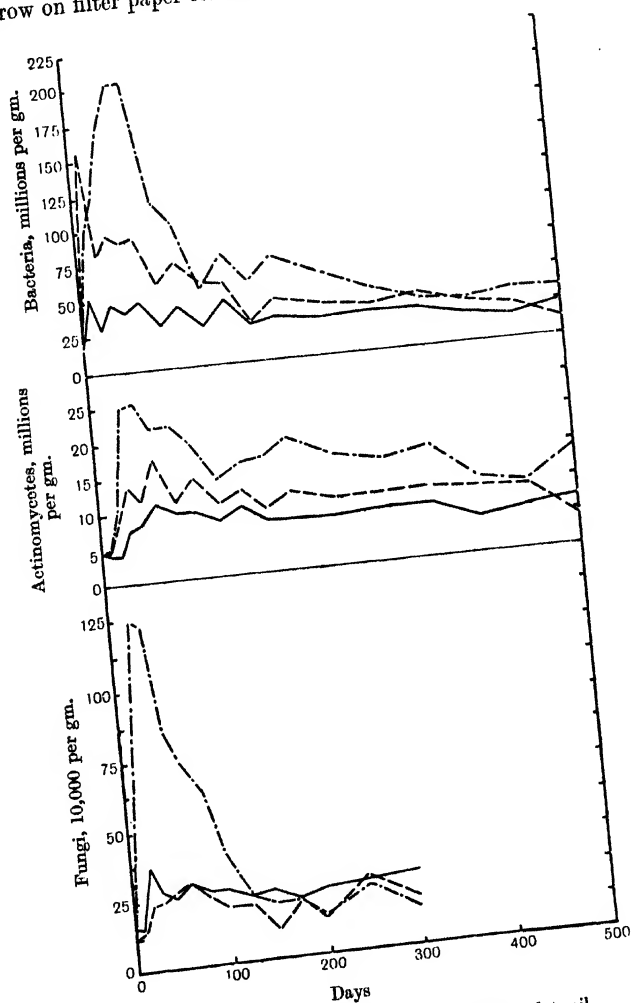
Fig. 1. Numbers of micro-organisms in neutral Park plot soil.
——— Control soil. – – – – Soil + manure. —·—·— Soil + manure + straw.

In the acid soil, conditions were very different. The bacterial numbers were very low and the addition of manure and straw did not give rise

to any considerable multiplication of bacteria or actinomycetes. It is, of course, conceivable that such organisms, specially adapted for the acid conditions and not capable of developing on the neutral agar medium, might have been active here; however, no striking development of such organisms took place on the acid agar used for counting fungi. The fungi were only slightly stimulated by the addition of manure, but in the soil with manure + straw their numbers at once rose to enormous heights
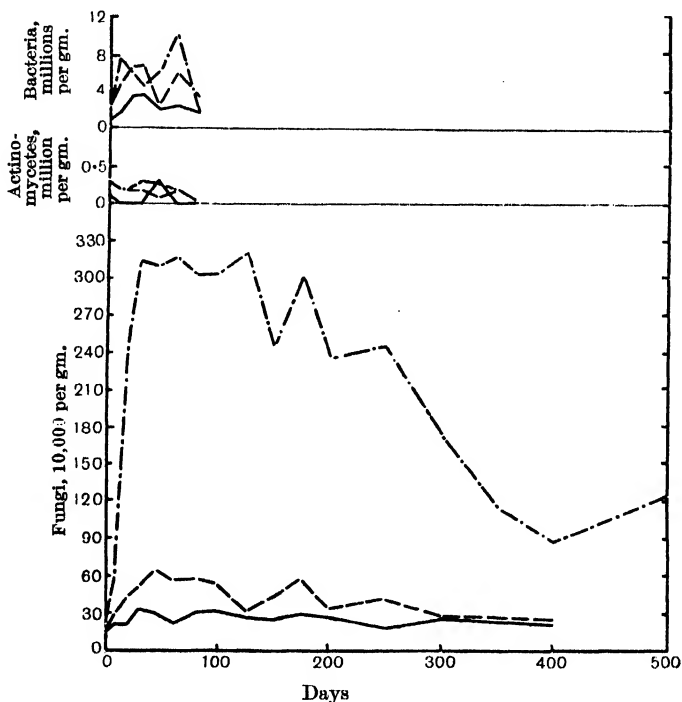
Fig. 2. Numbers of micro-organisms in acid Park plot soil.
———— Control soil.   – – – – Soil + manure.   —·—·— Soil + manure + straw.

and *remained constant* for a very long period. The most strongly multiplying forms were: *Trichoderma* sp. (Koningi?), which is an active cellulose-decomposing organism, *Zygorhynchus* sp. (Vuilleminii?), and a little yellow fungus, probably an *Amblyosporium*. When these experiments had run for some time there appeared a paper by McLennan (49), who developed a method for distinguishing between fungus spores and mycelium in the soil by drying the soil in vacuum; this treatment kills the vegetative mycelium, but leaves the spores intact. A count by this

method was carried out in these three soils after 400 days with the
following result:

	Fungi, thousands per gm.		
Soil	Fresh	Dried *in vacuo*	Remarks
Control	200	80	No *Trichoderma* in dried soil
Soil + manure	240	104	Very few *Trichoderma* in dried soil
Soil + manure + straw	880	380	Very few *Trichoderma* in dried soil

The fungus colonies on the plates apparently originated from spores
as well as from vegetative mycelium, but the *Trichoderma* (and *Zygo-rhynchus*) seem to be present mainly as mycelium, since they were most

Table I. *Chemical changes in Park plot soil, pH 7·0, with addition
of old farmyard manure and straw.*

Time	Control soil		Soil + manure		Soil + manure + straw	
	NO_3-N*	NH_4-N*	NO_3-N	NH_4-N	NO_3-N	NH_4-N
Start	0·0	0·4	0·0	1·0	0·0	1·0
After 100 days	0·8	0·0	0·8	0·0	1·2	0·0
„ 150 „	6·0	0·0	12·6	0·0	5·1	0·0
„ 200 „	8·4	0·0	14·0	0·0	2·4	0·0
„ 250 „	10·9	0·0	20·9	0·0	8·5	0·0
„ 300 „	13·3	0·0	24·9	0·0	12·8	0·0
„ 350 „	17·4	0·0	29·5	0·0	19·2	0·0
„ 400 „	18·9	0·0	31·1	0·0	21·5	0·0
„ 450 „	20·2	0·0	30·2	0·0	24·4	0·0
„ 500 „	19·9	0·0	32·5	0·0	24·7	0·0
Excess of NO_3-N over control, as % of added N	—		29·4 32·1		0·0 10·2	
α-humus as % of total dry matter:						
Start	2·19 with 4·11 N		2·47 with 3·93 N		2·38 with 4·08 N	
After 300 days	2·06 „ 4·00 N		2·43 „ 3·63 N		2·53 „ 3·51 N	
„ 500 „	1·81 „ 3·77 N		2·36 „ 3·38 N		2·53 „ 3·15 N	
Excess of α-humus N over control, as % of added N	—		18·1 14·5 30·6		14·5 14·1 24·2	
% total N:						
Start	0·355		0·394		0·404	
After 300 days	0·361		0·402		0·403	
„ 500 „	0·360		0·390		0·389	
% total C:						
Start	4·37		4·95		5·76	
After 300 days	4·01		4·42		4·67	
C:N ratio†:						
Start	12·3:1		12·6:1		14·3:1	
After 300 days	11·5:1		11·7:1		12·0:1	

* mg. of N per 100 gm. of air-dry soil.
† Mineral N subtracted.

strongly affected by the drying. The higher counts thus do not merely indicate that spore formation has taken place, but that there has been an active growth of fungi, resulting in a formation of considerable amounts of mycelium which is still living after 400 days.

The chemical changes taking place in the soils are shown in Tables I–II. The figures show that the accumulation of nitrate in the neutral control soil was very small for the first 100 days, probably because the soil was rich in grass roots and other plant residue poor in N. But in the interval from 100 to 150 days the nitrate production became active and proceeded regularly, gradually becoming slower, so that after 400–500 days about 200 parts per million of nitrate N accumulated. The soil + manure behaved somewhat similarly: no nitrate accumulated during the first 100 days, but from then the nitrate production proceeded fairly regularly until after 350 days, from which time very little more nitrate accumulated. At the end, the excess of nitrate over control corresponded to 32·1 per cent. of the manure N. In the soil with manure + straw the depression of nitrate formation was of course very marked, but wore off gradually, so that after 300 days the nitrate content about reached the level of the control soil. By this time the C:N ratio, originally 14·8:1, had been narrowed to 12·0:1, which is nearly the same as in the other two soils. From now on nitrate was produced in excess over control soil, but the nitrification remained less complete than in the case of manure alone. After 300 days the soil + straw had lost considerably more of its carbon than the other two soils, suggesting that the carbon of the straw is more easily attacked than that of the manure, as also found by Lemmermann *et al.* (35), Fraps (18), Potter and Snyder (59), and Lemmermann and Wiessmann (36). The soils thus gradually adjust themselves to the same C:N ratio, similar to that generally found in field soils. The total N determinations during the course of the experiment did not show any changes of undoubted significance. The humus determinations show that the amount of α-humus decreased slowly in the control soil, and that the N percentage of what remained fell from 4·11 to 3·77. In the soil + manure the amount of humus also decreased a little during the experiment, but remained constantly in excess over that of the control soil, and this excess was somewhat larger than the approximate 0·3 per cent. introduced with the manure. This suggests that either the added organic matter in these soils had protected the soil humus from attack, or else that some additional humus had been synthesised here. The same is the case in the soil with manure + straw, where the introduction of lignin with the straw further increased the

amount of humus a little. The percentages of N in humus in the two treated soils were lower than in the control soil, because the humus of the manure contains only 2·6 per cent. N. In these soils, as in the control, the N content of the humus fell during the storage, but by the end of the experiment the total humus N in the manured soil still exceeded that of the control soil by an amount equivalent to about 30 per cent. of the manure N added.

Table II. *Chemical changes in Park plot soil, pH 3·8, with addition of old farmyard manure and straw.*

Time	Control soil		Soil + manure		Soil + manure + straw	
	NO$_3$-N*	NH$_4$-N*	NO$_3$-N	NH$_4$-N	NO$_3$-N	NH$_4$-N
After 100 days	1·1	7·9	2·6	8·8	0·5	0·0
„ 150 „	0·3	7·8	2·1	8·1	0·0	1·4
„ 200 „	0·7	9·2	3·9	11·9	0·5	4·2
„ 250 „	0·0	12·8	1·4	16·0	0·0	6·6
„ 300 „	0·2	18·5	2·4	22·0	Trace	14·2
„ 350 „	0·2	18·7	1·4	24·2	0·0	15·6
„ 400 „	1·4	20·3	2·3	24·1	1·6	16·2
„ 500 „	1·9	18·2	2·2	24·9	1·7	17·7
Final excess of mineral N over control, as % of added N	—		17·8		0·0	
α-humus %: After 300 days	4·72		4·98		5·33	
% N in α-humus: After 300 days	3·37		3·30		3·15	
Final excess of α-humus N over control, as % of added N	—		13·3		18·0	

* mg. per 100 gm. of air-dry soil.

In the acid soils no determinations of ammonia and nitrate were made at the start of the experiment. From 100 days and onwards there was an accumulation of considerable amounts of ammonia and small amounts of nitrate in the control soil and the soil + manure, but the ammonification of the manure here proceeded much more slowly than its nitrification in the neutral soil, so that after 500 days the excess of ammonia + nitrate over control corresponded to only 17·8 per cent. of the manure N. In the soil with manure + straw the depression in the formation of mineral N was still more pronounced than in the neutral soil, as might be expected from the abundant development of fungi, but in this soil also the depression gradually wore off, and after 500 days the content of ammonia was nearly on a level with that of the control soil. In these soils too the humus of the manure persisted, and the

addition of straw gave a marked increase in humus content after 300 days, probably because the lignins did not undergo any noticeable decomposition in this extremely acid soil. It is noteworthy that in the third soil the excess in humus over soil + manure was 0·3 per cent., and the addition of 2 per cent. straw corresponds to an addition of approximately 0·4 per cent. lignin.

(3) *Second series of experiments.*

In this series the decomposition of fresh farmyard manure with and without extra addition of ammonium sulphate and straw in faintly acid soil with and without lime was studied.

The soil used for this experiment was a light sandy soil, rather poor in organic matter and of pH 6·0 from Woburn Experimental Station. The air-dry soil received 16 per cent. water and 0·2 per cent. $Ca(H_2PO_4)_2$ in order to make its reaction a little more acid. The soil contained at the start of the experiment (on air-dry basis): total C, 1·22 per cent.; total N, 0·112 per cent.; α-humus, 0·67 per cent. with 3·8 per cent. N. The following experiments were started:

(1) No addition (control)
(2) Manure corresponding to 2 per cent. dry matter
(3) Do. + 0·0538 per cent. $(NH_4)_2SO_4$
(4) Do. + Do. + 0·7 per cent. oat straw

All in two separate series with and without 1 per cent. $CaCO_3$

The manure was from a manure heap left loosely in the open. It contained 17·6 per cent. dry matter and 0·349 per cent. N, of which only a very small part was present as ammonia. The composition of the manure was on a basis of dry matter:

Total N	1·93 %	Ash	27·0 %
NH$_4$-N	0·06	α-humus	16·5
Total C	39·6	N in α-humus	2·2

The C:N ratio of the manure was thus 20·6:1. The addition of ammonium sulphate gave the manure a content of 2·50 per cent. N, corresponding to a C:N ratio of 15·8:1, and the further addition of straw with 0·48 per cent. N and 40·1 per cent. C restored approximately the original C:N ratio—21·6:1.

The soils were kept for 360 days at room temperature. The counts of micro-organisms are seen in Figs. 3 and 4, and the chemical changes in Tables III and IV.
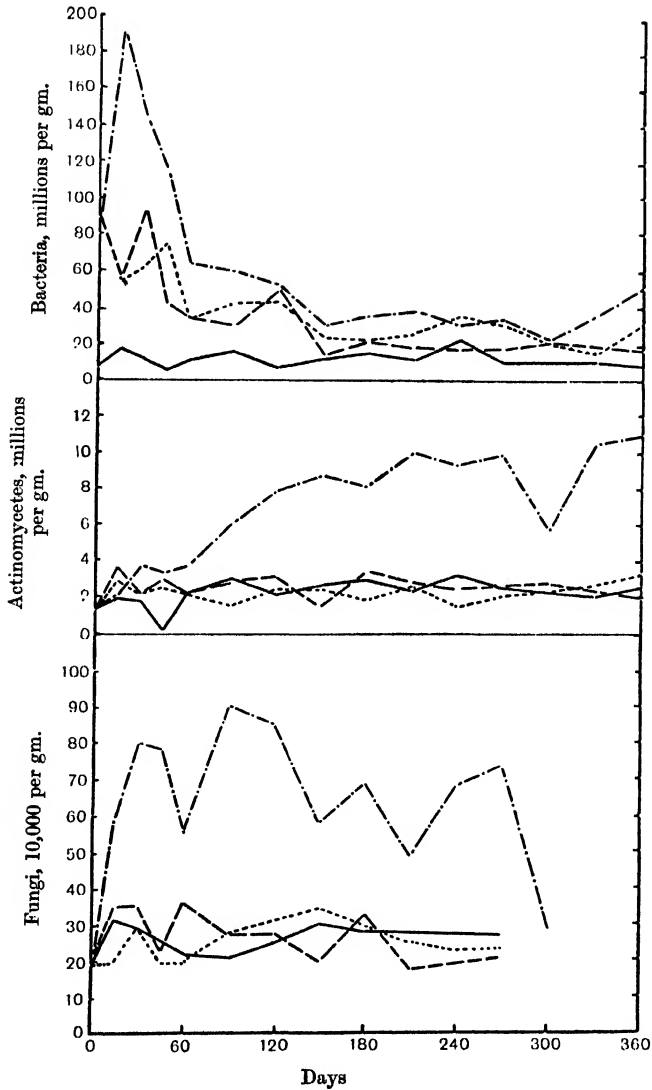
Fig. 3. Numbers of micro-organisms in Woburn soil, without CaCO₃.

——— Control soil. — — — Soil + manure. ······ Soil + Do. + (NH₄)₂SO₄.
—·—·— Soil + Do. + Do. + straw.

In the control soils the numbers of micro-organisms were quite low in this case, and the liming had no effect. In the soils with additions of manure the initial figures were high, on account of the large numbers of living organisms introduced with the fresh manure. In the soils with
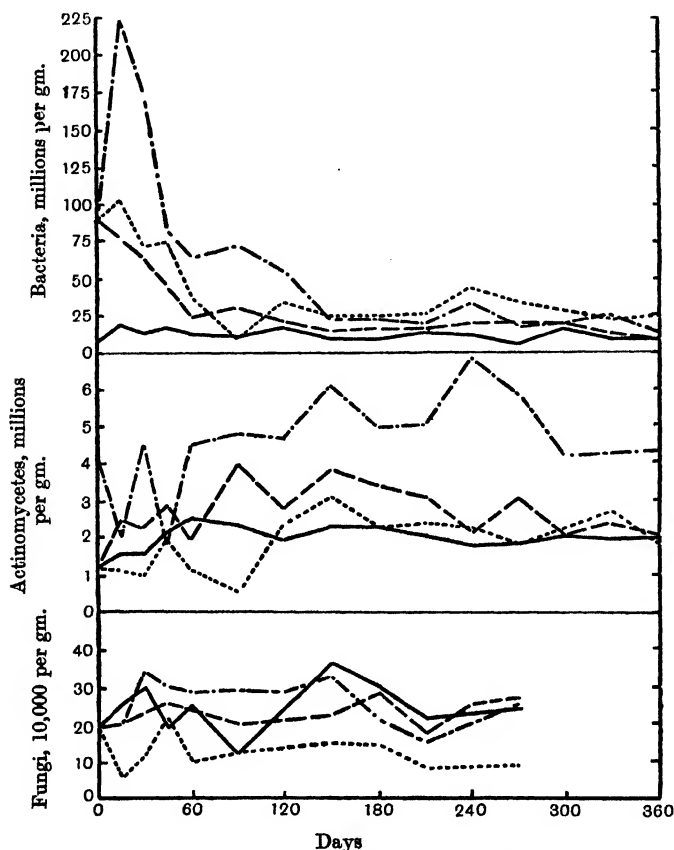
Fig. 4. Numbers of micro-organisms in Woburn soil + CaCO$_3$. Second series.
———— Control soil. – – – – Soil + manure. ⋯⋯⋯ Soil + Do. + (NH$_4$)$_2$SO$_4$.
—·—·— Soil + Do. + Do. + straw.

manure alone and with manure + extra N the figures fell gradually from the start, and after 90–150 days became about equal to those of the control soils, but the extra addition of straw caused the bacteria to multiply vigorously both in limed and unlimed soil, and not until after 200 days did the numbers go down to about the same range as in the control soils. The actinomycetes were not much affected by manure or

Table III. *Chemical changes in Woburn soil, unlimed, pH 6·0, with addition of farmyard manure, ammonium sulphate, and straw.*

Time	Control soil NO_3-N*	NH_4-N*	Soil + manure NO_3-N	NH_4-N	Soil + manure + $(NH_4)_2SO_4$ NO_3-N	NH_4-N	Soil + manure + $(NH_4)_2SO_4$ + straw NO_3-N	NH_4-N
Start	3·5	0·2	3·5	0·4	3·5	11·8	3·5	11·8
After 45 days	5·4	0·0	8·1	0·1	17·3	0·0	9·7	0·3
,, 90 ,,	6·5	0·0	9·2	0·0	21·3	0·0	9·5	0·0
,, 120 ,,	6·8	0·0	10·0	0·0	22·0	0·0	11·4	0·0
,, 150 ,,	7·0	0·0	10·3	0·0	21·6	0·0	12·6	0·0
,, 180 ,,	7·5	0·0	11·9	0·0	24·4	0·0	15·6	0·0
,, 210 ,,	7·1	0·0	11·9	0·0	25·0	0·0	17·1	0·0
,, 240 ,,	9·9	0·0	17·8	0·0	28·4	0·0	17·7	0·0
,, 270 ,,	8·7	0·0	16·8	0·0	28·0	0·0	16·1	0·0
,, 300 ,,	7·9	0·0	14·6	0·0	29·2	0·0	16·8	0·0
,, 330 ,,	8·7	0·0	17·1	0·0	31·4	0·0	16·8	0·0
,, 360 ,,	8·7	0·0	17·3	0·0	29·4	0·0	18·6	0·0
Final excess of NO_3·N over control, as % of added N	—		22·3		41·4		18·5	
α-humus % Start:	0·67		1·00		1·00		1·00	
After 360 days	0·66		0·93		1·07		1·10	
% N in α-humus: After 360 days	3·63		3·22		3·30		3·40	
% total N: Start	0·112		0·148		0·159		0·162	
End	0·110		0·147		0·159		0·160	
% total C: Start	1·22		2·01		2·01		2·29	
End	1·22		1·76		1·83		1·88	
% added C disappeared	—		25·9		16·5		34·5	
Final C:N ratio†	12·4:1		13·5:1		14·1:1		13·3:1	
pH: After 90 days	5·7		5·8		5·5		5·7	
,, 360 ,,	5·6		5·6		5·4		5·6	

* mg. per 100 gm. of air-dry soil. † Mineral N subtracted.

$(NH_4)_2SO_4$, but were stimulated by the addition of straw, especially in the later stages of the experiment. As in the previous series of experiments, the fungi were not affected by the addition of manure or ammonium sulphate, except for the fact that their numbers seemed to drop markedly in the limed soil + ammonium sulphate. The addition of straw, however, caused them to multiply vigorously in the unlimed soil, and for a considerable period. After 300 days most of the fungus colonies on the plates originated from vegetative mycelium, as was shown by a count carried out according to the method of McLennan[49]. This showed:

"Numbers" of fungi in fresh soil, per gm.   270,000

,,   ,,   dried   ,,   80,000

The increase in fungi in this soil was mostly due to one particular species, probably a *Monosporium*, which produced a vigorous growth on cellulose as filter-paper in mineral solution.

The chemical changes in this soil were very interesting. In the acid control soil the nitrate production followed a fairly smooth curve. In this soil with manure, nitrate was formed from the beginning, and, after 240 days, nitrate formation was marked, and subsequently maintained a fluctuating level so that at the end of the experiment the excess of nitrate over control corresponded to 22·3 per cent. of the N of the manure. In the soil with manure and ammonium sulphate the nitrification was stronger, and the excess over the previous soil was very nearly equal to the amount of N added as ammonium sulphate N. The curve ran very smoothly here, and the amounts of nitrate remained fairly constant after 240 days, so that at the end of the experiment the excess of nitrate over control corresponded to 41·4 per cent. of the total N added. This corresponds to all the added ammonium sulphate—24 per cent. of the manure N, almost the same as in the soil with manure alone.

The soil with manure + N + straw gave a nitrate content which, after 90 days and onwards, was almost the same as in the soil with manure alone, save that the rise occurred at a somewhat earlier date (180 days), and by the end of the experiment the nitrate corresponded to 18·5 per cent. of the added N. The fact that the C:N ratio of this material was somewhat higher than that of the manure alone perhaps explains the smaller percentage nitrification.

In the limed soils, the nitrate figures in the control soil were practically the same as those in the corresponding unlimed soil. The soil with manure showed a nitrate accumulation which, from 120 days onwards, was little different from that of the unlimed soil with manure (the figures fluctuated, but were on the same general level), excess of nitrate over control at the end of the experiment corresponding to 20·7 per cent. of the manure N. The soil with manure + N showed an increased nitrate accumulation which remained constant from 210 days onwards, but the increase was smaller than one would expect, and corresponded at the end of the experiment to only 26·8 per cent. of the total added N, or 6–7 per cent. of the manure N. Finally, in the soil with manure + N + straw the nitrate figures were almost identical with those from the soil + manure alone, so that at the end of the experiment the excess of nitrate over control corresponded to only 13·7 per cent. of the added nitrogen. The introduction of enough straw to restore the original C:N ratio was thus sufficient both in the unlimed and in the limed soil to prevent the nitrification of the added ammonium sulphate, but it is remarkable that

Table IV. *Chemical changes in Woburn soil, limed, with addition of farmyard manure, ammonium sulphate, and straw.*

Time	Control soil		Soil + manure		Soil + manure + (NH$_4$)$_2$SO$_4$		Soil + manure + (NH$_4$)$_2$SO$_4$ + straw	
	NO$_3$-N*	NH$_4$-N*	NO$_3$-N	NH$_4$-N	NO$_3$-N	NH$_4$-N	NO$_3$-N	NH$_4$-N
Start	3·5	0·2	3·5	0·4	3·5	11·8	3·5	11·8
After 45 days	5·9	0·0	7·0	0·2	16·9	0·0	9·0	0·0
,, 90 ,,	6·7	0·0	6·0	0·0	17·3	0·0	8·7	0·0
,, 120 ,,	7·0	0·0	9·2	0·0	19·9	0·0	9·5	0·0
,, 150 ,,	7·1	0·0	11·6	0·0	19·8	0·0	11·3	0·0
,, 180 ,,	7·2	0·0	12·3	0·0	21·4	0·0	13·9	0·0
,, 210 ,,	7·4	0·0	14·8	0·0	22·0	0·0	15·6	0·0
,, 240 ,,	7·8	0·0	14·2	0·0	28·0	0·0	14·6	0·0
,, 270 ,,	8·5	0·0	16·5	0·0	22·1	0·0	16·0	0·0
,, 300 ,,	8·4	0·0	19·4	0·0	22·4	0·0	17·1	0·0
,, 330 ,,	8·4	0·0	17·2	0·0	23·0	0·0	17·1	0·0
,, 360 ,,	8·7	0·0	16·7	0·0	22·1	0·0	16·0	0·0

	Control soil	Soil + manure	Soil + manure + (NH$_4$)$_2$SO$_4$	Soil + manure + (NH$_4$)$_2$SO$_4$ + straw
Final excess of NO$_3$-N over control, as % of added N	—	20·7	26·8	13·7
α-humus %:				
Start	0·67	1·00	1·00	1·00
After 360 days	0·53	1·02	0·85	0·93
% N in α-humus:				
After 360 days	3·80	3·47	3·58	3·48
% total N:				
Start	0·111	0·146	0·157	0·160
End	0·102	0·144	0·147	0·141
% total C (excl. of carbohydrates):				
Start	1·22	1·95	1·95	2·23
End	1·22	1·74	1·62	1·73
% added C disappeared	—	26·5	41·7	46·3
Final C:N ratio†	13·2:1	13·7:1	12·9:1	13·8:1
pH:				
After 90 days	6·7	6·7	6·5	6·6
,, 360 ,,	6·1	6·3	6·1	6·2

* mg. per 100 gm. of air-dry soil.   † Mineral N subtracted.

the nitrification seemed less complete in the limed than in the unlimed soil. The determination of the total N at the end of the experiment suggests an explanation of this. In the acid soil series there were no significant changes in the N content, but the limed soil showed a loss which is considerable in the soil with manure + ammonium sulphate + straw. When we allow for this loss, we see that the amounts of N *left untransformed* were considerably larger in the acid soils. Thus we have here a real parallel to the losses of N in manured soils under field conditions: the drop in N content of the last soil—from 0·160 to 0·141 per cent.—corresponds to a loss of 35 per cent. of the added N during one year.

It is difficult to explain what may have been the cause of this loss, but it is not impossible that denitrification may have played a rôle,

although the soil was but moderately moist and well aerated, and no large amounts of nitrate accumulated until the readily available energy material in the soil had been exhausted, as can be seen from the drops in the bacterial numbers. It must be remembered, however, that several experiments have shown that very slowly decomposible organic material (Nolte(53)) and soil humus (Oelsner(54)) may serve as sources of energy for denitrification, and experiments by Arndt(2) have shown a loss of N, probably due to denitrification, in limed high-moor soil which was well aerated and only moderately moist. This harmonises well with the fact that Winogradsky(86) has found obligate anaerobic *Clostridia* developing in their vegetative state in soil far from saturated with water. That the losses occur only in limed soils agrees with the fact that the denitrifying bacteria, as shown by Sacharowa(68), are very sensitive to acidity and stop their activity at pH 5·9–6·1.

A small additional experiment was carried out with the residue of unlimed soil + manure after the experiment had been discontinued. Two portions of 100 gm. of air-dry soil received each 17·0 per cent. water, one of them also 1 per cent. $CaCO_3$, and were kept at 25° C. for 25 days. Determinations of total N and nitrate N then showed:

	Soil without $CaCO_3$	Soil + $CaCO_3$
NO_3-N, mg. per 100 gm.	19·6	19·0
Total N, % at start	0·147	0·146
Total N, % at end	0·148	0·143

There was no significant change in the N content of either soil. An attempt was made to get an idea of whether there was a higher number of denitrifying bacteria in the latter soil. A dilution experiment in Giltay's solution was carried out with the following result (incubation for 7 days at 25° C.):

Dilution	Tube no.	Fermentation in solution inoculated with	
		Soil without $CaCO_3$	Soil with $CaCO_3$
1:100	a	+	+
	b	+	+
1:1000	a	+	+
	b	+	+
	c	–	+
	d	–	+
1:10,000	a	–	+
	b	–	–
	c	–	–
	d	–	–

There is an indication that denitrifying bacteria are a little more numerous in the limed soil, though the loss of N produced by them was insufficient to give a significant result by analysis. It would probably be

worth while to undertake a closer study of the conditions under which denitrifying bacteria are able to cause a loss of N from the soil, for it seems possible that the importance of denitrification in the soil has been first exaggerated and then underestimated. It should be noted that the pH determinations in the soils show possibility of ammonia evaporation to be out of question.

The humus determinations from the Woburn soil show that the manured soils have 0·27–0·44 per cent. in excess over the control soils, and their humus is somewhat poorer in N than that of the control soils. The manure has originally introduced 0·33 per cent. humus with 2·2 per cent. N, which seems to have persisted fairly completely during the whole experiment. This amount has of course been further increased by the addition of lignin in the straw. The total C determinations show that, in the two control soils, the losses in C are within the limits of the analytical error—in accordance with the low numbers of micro-organisms in these soils—and the losses of manure C are not very large in the un-limed soils. The soils with addition of straw have lost most, as in the previous experiment. In the limed soils with manure and extra N the losses in C are considerable, so that, owing to the losses of N in the limed soils, the C:N ratios tend to become equal in all soils, namely 12·2–14·1:1, a somewhat wide ratio.

(4) *Third series of experiments.*

In this series a comparison between two manures poor and rich in nitrogen in heavy clay soil was studied.

In order to study the influence of the C:N ratio of the manure, an experiment was carried out with two manures, of which one was very poor, the other very rich in N. The soil was a heavy clay soil from Hoosfield, fairly rich in organic matter and of pH 6·3. It contained in air-dry condition: total N, 0·165 per cent.; total C, 1·84 per cent. The N-poor manure was the same as in the previous experiment, the N-rich, a sample of manure treated by the Edelmist process, which has in recent years attracted considerable attention in Germany[1]. It contained

Dry matter (%) ...	...	...	...	22·4
Total N, % of dry matter		...	...	3·39
NH_4-N	,,	...	...	0·34
Total C	,,	...	...	43·4
Ash	,,	...	...	20·5
α-humus	,,	...	...	27·5
N in α-humus	,,	...	...	2·97

[1] The manure was supplied through the courtesy of Gärstatt G. m. b. H., Munich, Germany.

The manures were used in the same quantities as before—corresponding to 2 per cent. of dry matter, and flasks were kept at room temperature for 360 days.
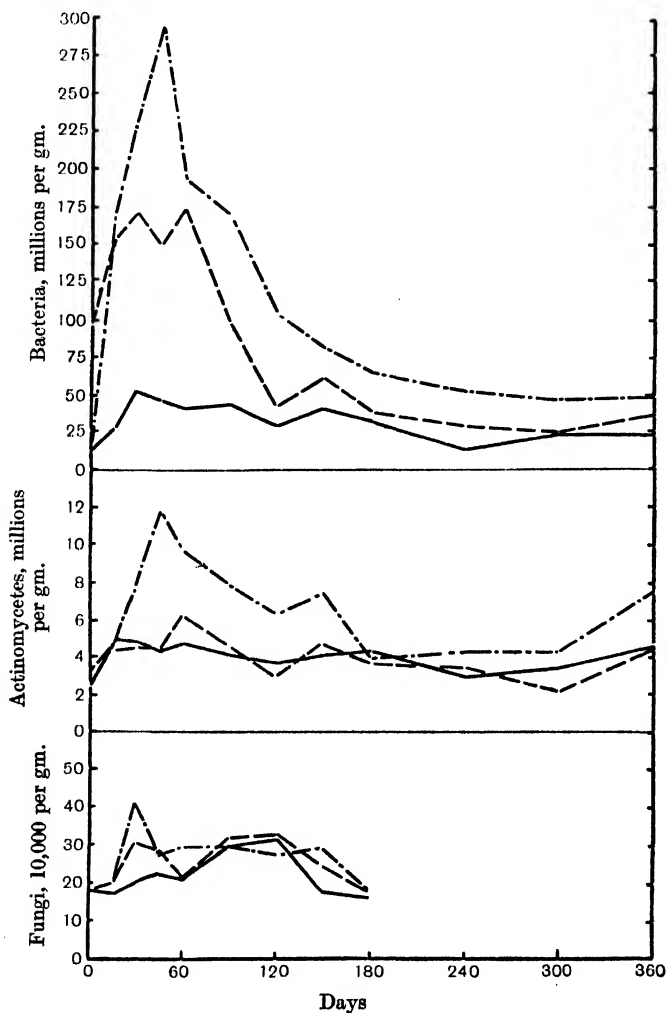
Fig. 5. Numbers of micro-organisms in Hoosfield soil. Third series.

———— Control soil.   – – – Soil + fresh manure.   —·—·— Soil + Edelmist.

The counts of bacteria, actinomycetes and fungi are shown in Fig. 5. The control soil had quite high numbers of bacteria from 30 to 90 days, after which time the numbers fluctuated on a lower level. The soil +

manure had a high number of bacteria at the start, and this increased greatly during the first 15–60 days, after which time a decrease set in, so that from 120 days the numbers remained practically the same as in the control soil. The Edelmist was poor in living organisms, so that this soil started with a low number of bacteria, but they multiplied rapidly, reaching very high numbers by the 45th day, after which they fell greatly, but still remained in excess over the other two soils. The actinomycetes were but slightly affected by the addition of fresh manure; in the soil with Edelmist they were somewhat stimulated, but not to the same extent as the bacteria. As in the previous experiments, the fungi were not affected to any significant extent.

Table V. *Chemical changes in Hoosfield soil, pH 6·3, with addition of fresh farmyard manure and Edelmist.*

Time	Control soil		Soil + fresh farmyard manure		Soil + Edelmist	
	NO₃-N*	NH₄-N*	NO₃-N	NH₄-N	NO₃-N	NH₄-N
Start	0·6	0·0	0·6	0·2	0·6	6·8
After 30 days	5·0	0·0	3·3	Trace	9·5	1·2
,, 60 ,,	7·8	0·0	5·6	0·0	10·1	0·0
,, 90 ,,	8·7	0·0	7·3	0·0	11·6	0·0
,, 120 ,,	9·2	0·0	9·7	0·0	13·6	0·0
,, 150 ,,	10·2	0·0	11·1	0·0	18·7	0·0
,, 180 ,,	10·7	0·0	13·1	0·0	19·5	0·0
,, 240 ,,	10·1	0·0	14·4	0·0	21·4	0·0
,, 300 ,,	10·0	0·0	15·4	0·0	23·2	0·0
,, 360 ,,	12·0	0·0	17·4	0·0	26·5	0·0
Final excess of NO₃ over control, as % of added N	—		13·6		22·9	
α-humus %:						
After 180 days	0·79		1·13		1·38	
,, 360 ,,	0·64		1·09		1·34	
% N in α-humus:						
After 180 days	3·46		3·66		3·63	
,, 360 ,,	3·77		3·56		3·62	
Excess of α-humus N over control, as % of added N:						
After 180 days	—		36·5		33·7	
,, 360 ,,	—		38·1		35·8	
% total N:						
Start	0·165		0·200		0·227	
After 360 days	0·185		0·216		0·239	
% total C:						
Start	1·84		2·58		2·66	
After 180 days	1·70		2·21		2·41	
% added C disappeared:						
After 180 days	—		46·7		28·8	

* mg. per 100 gm. of air-dry soil.

The chemical changes are found in Table V. The control soil showed a gradual accumulation of nitrate, whereas in the soil with fresh manure a marked depression in nitrate formation took place, and lasted for 120 days. The depression was then overcome, and a slow production of nitrate from the manure set in, so that after 300–360 days the excess of nitrate over the control soil corresponded to 13·6 per cent. of the manure N. The Edelmist soil started with a high content of ammonia N, which was nitrified after 30–60 days, but the excess over control was much less than should have been produced by oxidation of the ammonia N added with the manure. There was thus here a relative depression of nitrate formation, which lasted for about 150 days, after which time the amount of nitrate rose, first rapidly, then more slowly, so that at the end of the experiment the excess over control corresponded to 23 per cent. of the manure N. The Edelmist thus showed a nitrifiability much superior to that of ordinary farmyard manure, but the absolute amount of N left untransformed was also larger in the case of Edelmist. It is therefore doubtful whether one is justified in regarding the N compounds of the Edelmist as particularly easily available to the soil micro-organisms—an opinion which has repeatedly been expressed by German investigators, *e.g.* Löhnis [44] and Ruschmann [63]. It seems rather that the Edelmist owes its superior value merely to its higher percentage of N, that is, to its more favourable C:N ratio. The humus determinations show that, after 180 and 360 days, the two manured soils had an excess over control in both humus and humus N even larger than that which corresponds to the humus introduced with the manure. These N compounds thus appear to have been perfectly resistant to the attack of micro-organisms for a period of 6–12 months, and a small amount of extra humus seems to have been formed. The N determinations show a rather peculiar phenomenon: N fixation took place in all soils, most in the control and least in the soil with Edelmist.

(5) *Fourth series of experiments.*

In this series a comparison between natural farmyard manure and synthetic farmyard manure made by the "Adco" process (Hutchinson and Richards [28]) was studied.

For this experiment a heavy clay soil, poor in organic matter and of *p*H 6·4, from an unfertilised plot on Hoosfield, was used. It contained an air-dry basis: total N, 0·094 per cent.; total C, 0·0881 per cent.; humus, 0·23 per cent. with 4·59 per cent. N. The natural manure was well fermented and had been well stored. The synthetic manure, prepared from

wheat straw and ammonium sulphate, was well decomposed. The composition of the two manures was as follows:

	Natural manure	Synthetic manure
Dry matter (%)	21·5	20·2
Total N, % of dry matter	2·25	2·54
NH_4-N ,,	0·17	0·03
Total C ,,	38·2	36·5
Ash ,,	21·2	29·1
Humus ,,	17·9	20·7
N in α-humus ,,	2·30	2·46
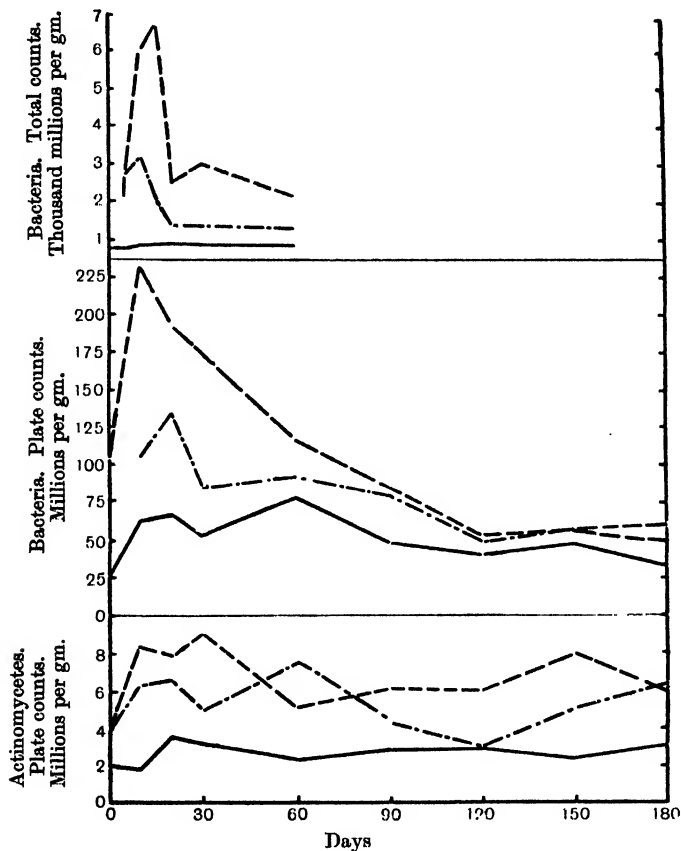
Fig. 6. Numbers of micro-organisms in Hoosfield soil. Fourth series.
——— Control soil. — — — Soil + nat. manure. —·—·— Soil + synthetic manure.

The manures were added in amounts corresponding to 2 per cent. dry matter, and flasks were kept at room temperature for 180 days. In this series bacterial numbers were estimated both by the plate method

and by the microscopical method developed by Gray and Thornton[22] in this laboratory.

The bacterial counts in Fig. 6 show that the absolute numbers of bacteria found by direct counting were on an average 20 to 30 times as high as those obtained by the plate method, but the initial rise and subsequent fall in bacterial numbers due to addition of manure are reflected in the figures obtained by both methods. The natural farmyard manure caused a very large increase 10–20 days after the addition of the manure. The actinomycetes were also somewhat stimulated by both kinds of manure.

Table VI. *Chemical changes in Hoosfield soil, pH 6·4, with addition of natural and artificial farmyard manure.*

Time	Control soil		Soil + natural farmyard manure		Soil + synthetic farmyard manure	
	NO_3-N*	NH_4-N*	NO_3-N	NH_4-N	NO_3-N	NH_4-N
Start	1·4	0·4	1·4	3·8	1·4	0·9
After 10 days	3·9	0·0	5·7	Trace	6·7	0·0
,, 20 ,,	4·3	0·0	5·3	0·0	7·8	0·0
,, 30 ,,	4·5	0·0	5·4	0·0	7·7	0·0
,, 60 ,,	5·7	0·0	5·3	0·0	14·0	0·0
,, 90 ,,	4·5	0·0	6·4	0·0	9·8	0·0
,, 120 ,,	4·6	0·0	6·7	0·0	9·5	0·0
,, 150 ,,	4·6	0·0	6·6	0·0	—	—
,, 180 ,,	4·0	0·0	7·0	0·0	9·8	0·0
Excess of NO_3-N over control, as % of added N	—		6·7		11·4	
α-humus %: After 180 days	0·23		0·65		0·73	
% N in α-humus	3·91		3·29		3·10	
Excess of α-humus N over control, as % of added N	—		27·8		23·1	
% total N: Start	0·094		0·136		0·142	
End	0·094		0·136		0·147	

* mg. of N per 100 gm. of air-dry soil.

The chemical changes are seen in Table VI. The natural manure underwent a slow nitrification in this soil; after 180 days the excess in nitrate N was not even as large as the initial addition of ammonium N. The synthetic manure gave rise at once to a vigorous nitrate formation, so that after 60 days the excess of nitrate corresponded to 16 per cent. of the total N (nearly all organic). After this period the process did not go any further, and there was even a decrease in nitrate content, the cause of which is rather obscure, but which has also been observed by

other workers, *e.g.* Glathe (20) and Scheibe (69). The humus determinations again show that the humus of both kinds of manure persisted in the soil, and some more seems to have been formed. The excesses in humus over control soil were 0·42 and 0·50 per cent. for natural and synthetic manure, the quantities added being 0·36 and 0·41 per cent. The amounts of N in humus in the manures amounted to 17·3 and 20·1 per cent. of total N, whereas the excesses in humus N at end of experiment corresponded to 28 and 23 per cent. of the added N. The determinations of total N show that in this case there was no loss of nitrogen.

In these four sets of experiments there was a formation of ammonia and nitrate from the organic N compounds of different kinds of farmyard manure in all cases but two. The first exception was with the strongly acid Park plot soil with manure + straw, in which an abundant fungus flora arose, the second was in the last experiment in which Hoosfield soil with manure was kept for only 180 days. The general results, however, are sufficient to disprove the general validity of the statement of Barthel and Bengtsson (6), that only the ammonium N of the farmyard manure is available for nitrification during the first year. Especially convincing in this respect is the first series of experiments—old farmyard manure in neutral Park plot soil. In this well-decomposed manure, in which practically all N is present in organic compounds, no less than one-fifth of the N has been nitrified after 300 days. In the case of fresh manure it might be argued that the amount of N which underwent nitrification might have been transformed into ammonia, if the manure had been allowed to ferment properly, and the Edelmist may not be entirely comparable with the ordinary farmyard manure. That the C:N ratio of the manure exerts a marked influence on the rate of nitrification can be seen from the following summary:

Series	Material	Time (days)	C:N of added material	% of N nitrified or ammonified
1 A (neutral)	Old manure	500	14·4 : 1	32
Do.	Do. + straw	500	28·3 : 1	10
1 B (acid)	Old manure	500	14·4 : 1	16
Do.	Do. + straw	500	28·3 : 1	None
2 A (unlimed)	Fresh manure	360	20·6 : 1	22
Do.	Do. + $(NH_4)_2SO_4$	360	15·9 : 1	41
Do.	Do. + Do. + straw	360	21·6 : 1	18
2 B (limed)	Fresh manure	360	20·6 : 1	21
Do.	Do. + $(NH_4)_2SO_4$	360	15·9 : 1	27
	Do. + Do. + straw	360	21·6 : 1	15
3	Fresh manure	360	20·6 : 1	14
Do.	Edelmist	360	12·8 : 1	23
4	Natural manure	180	16·9 : 1	7
Do.	Synthetic manure	180	15·1 : 1	11

The widening of the C:N ratio means an increased energy supply relatively to the supply of N; this causes the bacteria and other micro-organisms to multiply vigorously and to use up more N for the synthesis
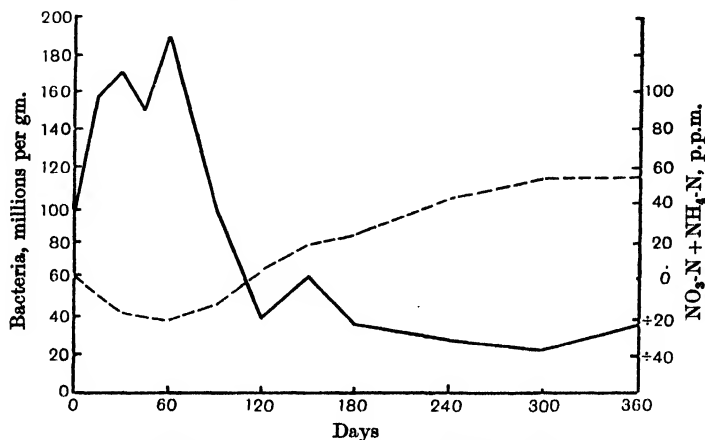
Fig. 7. Bacterial numbers and production of mineral N from farmyard manure in Hoos-field soil.

————— Bacterial numbers.   — — — Excess in mineral N over control soil.
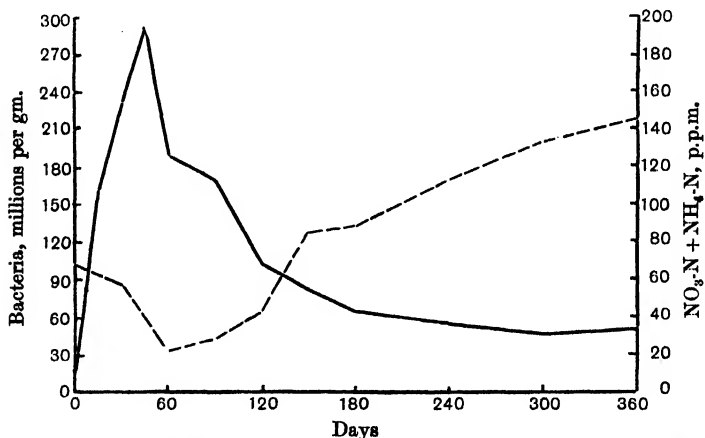
Fig. 8. Bacterial numbers and production of mineral N from Edelmist in Hoosfield soil.
————— Bacterial numbers.   — — — Excess in mineral N over control soil.

of their cell substance; not until the death of the cells can this N again be released and transformed into ammonia and nitrate, and in accordance with this we see from Figs. 7–13 *that there is an inverse relationship between bacterial numbers and amounts of ammonium + nitrate N.* In the

Woburn soil without straw, where the bacteria do not multiply much, this phenomenon is least marked, and in the acid Park plot soil, where the bacteria are almost inactive, the fungi show a similar relationship.
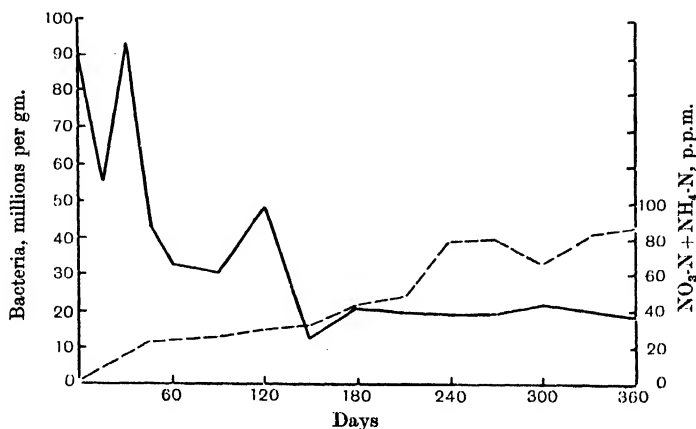
Fig. 9. Bacterial numbers and production of mineral N from farmyard manure in Woburn soil, unlimed.

———— Bacterial numbers.   — — — Excess in mineral N over control soil.
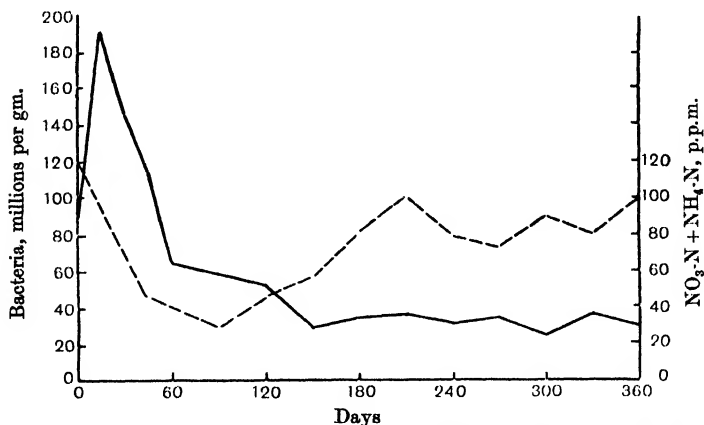
Fig. 10. Bacterial numbers and production of mineral N from manure + $(NH_4)_2SO_4$ + straw in Woburn soil, unlimed.

———— Bacterial numbers.   — — — Excess in mineral N over control soil.

If the manure is poor in N (wide C:N ratio) the content of nitrate in the soil is depressed below that of the control soil for a certain period, and even if it is richer in N, a marked formation of nitrate does not appear

at once, but begins when the bacterial numbers have again gone down to a low level or some time after this—probably at the time when the dead bacterial cells are in their turn undergoing decomposition. A similar
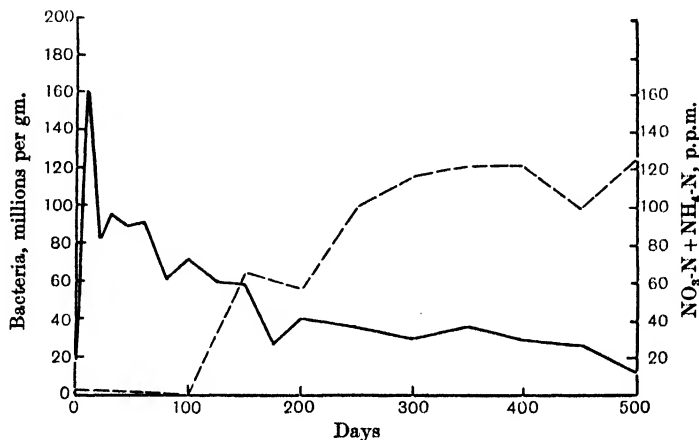
Fig. 11. Bacterial numbers and production of mineral N from farmyard manure in neutral Park plot soil.

——— Bacterial numbers.   — — — Excess in mineral N over control soil.
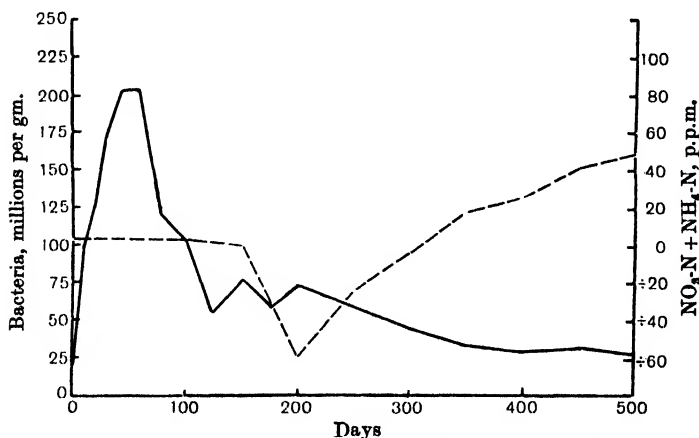
Fig. 12. Bacterial numbers and production of mineral N from farmyard manure + straw in neutral Park plot soil.

——— Bacterial numbers.   — — — Excess in mineral N over control soil.

inverse relationship between bacterial numbers and amounts of mineral N can be seen from the data given by Russell and Hutchinson (64), Joshi (31), and Wilson (85).

Although the higher nitrogenous compounds of manure do undergo decomposition, their mineralisation is very slow and incomplete. What is the cause of this? The idea at once suggests itself that, since the excesses in bacterial numbers over control soils continue for a long period, their total numbers might remain so high that the quantity of cell
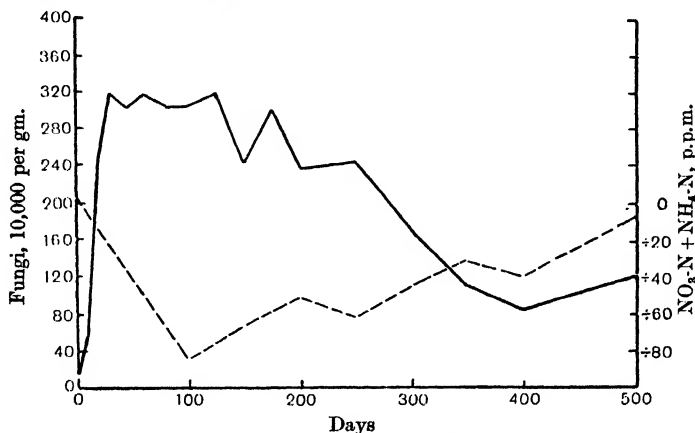
Fig. 13. "Numbers" of fungi and production of mineral N from farmyard manure + straw in acid Park plot soil.

———— "Numbers" of fungi.   — — — Excess in mineral N over control soil.

material would account for a considerable part of the manure N locked up at the end of the experiment. A glance at the total counts after 60 days (Fig. 6), however, is sufficient to dispose of this idea.

Hoosfield control soil   766 millions of bacteria per gm.
Soil + natural manure   2204   ,,   ,,
Soil + synthetic manure   1309   ,,   ,,

A total count in soil from Woburn (second series, unlimed) at the end of the experiment showed in:

Control soil   1560 millions of bacteria per gm.
Soil + manure   2660   ,,   ,,
Soil + Do. + $(NH_4)_2SO_4$ 1950   ,,   ,,
Soil + Do. + Do. straw   2460   ,,   ,,

We have here excesses over control soils corresponding to 400–1500 million cells per gm. of soil. This quantity can be roughly estimated to occupy 40–150 mm. per 100 gm. of soil, assuming that each bacterial cell has a cubic content of 1μ. If we reckon with a specific gravity of 1 for the bacterial cells, this quantity will be 40–150 mg. per 100 gm. of moist

soil, or 50–200 mg. per 100 mg. of air-dry soil. If we assume that the bacterial cells contain 20 per cent. dry matter with 10 per cent. N, we find 1–4 mg. of bacterial N in excess over the control soil. But the quantities of unnitrified manure N at the end of the experiments amount to 30–42 mg., of which that of the bacterial cells thus constitutes only a very small fraction. It is thus evidently not here, in the living bacterial matter, that we have to look for the untransformed and slowly transformable N of the manure. However, the total numbers of bacteria shortly after the addition of manure are very high; for instance, the total counts after 10 days in the Hoosfield soil + natural manure (Fig. 6) show an excess in bacteria over control soil, corresponding to nearly 6000 million cells per gm. of soil; this corresponds per 100 gm. of soil to 100–120 mg. of bacterial dry matter, or 10–12 mg. of N, which is 22 to 25 per cent. of the total amount of N added in the manure. This fact, that a not insignificant part of the manure nitrogen passes into the protoplasm of bacteria, introduces the question of decomposition of dead microbial protoplasm and the possible formation of more or less undecomposable humus-like nitrogenous compounds from this source (Waksman (82)). The N present in the humus fraction of the manure persists in the soil for 180 to 360 days, and sometimes some extra humus is formed. This considerable fraction of the manure N appears to be in a very slightly decomposable state, as the control experiments below also show.

(6) *Decomposition of manures in sand.*

In all the previous decomposition experiments we have relied, as is customary, upon the assumption that the differences in the contents of nitrate, humus, carbon, etc., between a control soil and soil with addition of some organic material, indicate the extent to which the organic material has been attacked. We are, in other words, assuming that the soil's own organic matter is attacked equally in both cases. How far this is true is not, however, known with certainty, and it is not at all unlikely that the addition of organic matter to the soil might protect the soil's organic matter from being attacked. In other cases it seems, as demonstrated by Löhnis (43), that the stimulation of the soil micro-organisms due to addition of organic material involves a greater attack on the soil organic matter itself. To have a control upon this, some decomposition experiments were carried out in sand, a medium which does not in itself contain any matter, from which ammonia or nitrate can be formed, and from which therefore the total production of nitrate becomes an index of the decomposition of the organic material. The following materials

were used: (1) Air-dried fresh farmyard manure. (2) Edelmist. (3) Old, badly stored farmyard manure. (4) Humus-free extraction residue of fresh farmyard manure, prepared by treating the manure several times with boiling 4 per cent. NaOH, until a nearly colourless extract was obtained; boiling the residue with 2 per cent. H_2SO_4; washing and drying. The air-dry materials had the following composition:

	Total N (%)	NH$_4$-N (%)	Total C (%)	Ash (%)
Fresh farmyard manure	1·85	0·01	37·6	25·6
Edelmist	2·86	0·11	42·2	19·3
Old farmyard manure	2·10	0·00	36·6	27·6
Extraction residue	1·23	0·00	37·7	—

The materials were added in quantities of 2 per cent. of dry matter to 300–400 gm. of sand; this mixture was moistened with 12 per cent. of distilled water including 1 c.c. of a suspension of garden soil (1:10) in distilled water. The flasks were kept at 25° C. for 90 days.

Table VII. *Nitrification of various manures in sand.*

Time	Fresh farmyard manure NO$_3$-N*	NH$_4$-N*	Edelmist NO$_3$-N	NH$_4$-N	Old farmyard manure NO$_3$-N	NH$_4$-N	Extraction residue of farmyard manure NO$_3$-N	NH$_4$-N
Start	0·0	0·2	0·0	2·2	Trace	0·0	0·0	0·0
After 20 days	4·5	0·6	7·8	0·6	3·1	Trace	0·0	0·0
„ 40 „	5·3	0·0	9·4	0·0	3·4	0·0	0·0	0·0
„ 60 „	6·1	0·0	13·4	0·0	4·2	0·0	0·0	0·0
„ 90 „	7·3	0·0	12·6	0·0	4·1	0·0	Trace	0·0
N added, mg. per 100 gm. of sand	37·0		57·2		42·0		24·6	
C added %	0·752		0·844		0·732		0·754	
C:N ratio, initial	20·3:1		14·8:1		17·4:1		30·6:1	
% of added N nitrified	19·7		22·0		9·8		0·0	
α-humus %: After 90 days	0·33		0·40		0·31		0·28	
% N in α-humus	1·93		2·42		2·16		2·76	
% of added N found in α-humus: After 90 days	16·6		16·8		16·0		—	
Total N, mg. per 100 gm. of sand†: After 90 days	40·2		55·8		41·4		—	
Total C %†: After 90 days	0·426		0·495		0·614		—	
C:N ratio, final‡	12·9:1		11·5:1		14·8:1		—	
% added C disappeared	41·3		39·3		14·1		—	

* mg. per 100 gm. of sand.
† Sand in a control experiment contained 1·6 mg. N and 25 mg. C per 100 gm. This quantity is subtracted here.
‡ NO$_3$-N subtracted.

The results of the analyses are seen in Table VII. All the manures underwent nitrification, which, as in the soils, proceeded with decreasing rapidity, so that after 3 months 10–22 per cent. of the manure N was nitrified. The Edelmist and fresh manure showed rapid nitrate production, whereas the old manure, in spite of its more narrow C:N ratio, showed a much slower nitrification. The explanation is probably that the C:N ratio of the manure must be reduced to a certain value, usually 10–12:1, before nitrification can start, and this value was reached more slowly in the case of old manure, which consisted chiefly of old resistant residues.

The humus determinations showed a little decrease, though perhaps not significant, in humus N in fresh manure and in old manure, and, especially in Edelmist, the decomposition of this fraction appeared to be definite, though not considerable. The total N determinations show that none of the sand-manure mixtures lost significant quantities of N.

The manure residue with its wide C:N ratio did not undergo any nitrification, but the humus determination shows the interesting fact that a considerable amount of humus, containing 2·8 per cent. N, was formed from this material, which at the start did not contain any alkali-soluble material. It would probably be premature to claim that this humus was synthesised by micro-organisms active in the sand. The lignins are only partly soluble in alkali, and it is possible that the humus found here was mostly derived from lignin which, during the incubation, perhaps under the influence of micro-organisms, became soluble in alkali.

(7) *Decomposition of humus.*

Finally, an experiment was carried out in order to compare the decomposition of humus from manure and from soil. The manure humus was prepared from air-dry manure and Edelmist by extraction with boiling 4 per cent. NaOH, precipitation of the filtrate with H_2SO_4, boiling the precipitate in excess of H_2O (about 2 per cent. solution), filtering, washing till free from acid, and drying. The soil humus was a-humus from a fertile, loamy garden soil. The composition of the materials was:

	Total N (%)	Total C (%)	Methoxyl (%)	Ash (%)	Moisture (%)
Fresh manure humus	3·11	55·7	5·65	0·4	6·3
Edelmist humus	3·66	59·0	5·88	1·2	6·1
Soil humus	3·97	52·2	3·16	1·3	6·2

Two per cent. of the two sorts of manure humus and 1 per cent. of soil humus were added to 150 gm. portions of sand which further received

0·5 per cent. $CaCO_3$ and 12 per cent. of a 0·1 per cent. solution of K_2HPO_4 + 1 c.c. of a suspension of garden soil. The mixture was kept in round flasks of about 300 c.c. capacity for 240 days at 25° C.

Table VIII. *Decomposition of humus from manure, Edelmist and soil in sand culture.*

Time	Manure humus (2 %)		Edelmist humus (2 %)		Soil humus (1 %)	
	NO_3-N*	NH_4-N*	NO_3-N	NH_4-N	NO_3-N	NH_4-N
Start	0·0	0·0	0·0	0·0	0·0	0·0
After 60 days	Trace	Trace	0·0	0·0	0·0	Trace
„ 120 „	0·0	0·0	0·0	0·0	2·7	0·0
„ 180 „	0·0	0·0	2·3	0·0	4·7	0·0
„ 240 „	0·0	Trace	2·5	Trace	5·0	0·0
% of N nitrified	None		3·4		12·6	
% C at start	1·11		1·18		0·52	
% C at end	0·86		1·07		0·44	
C:N initially	17·9:1		14·8:1		13·1:1	
C:N finally (min. N subtracted)	13·8:1		15·2:1		12·7:1	

* mg. per 100 gm. of sand.

Table VIII shows that the soil humus underwent a slow, but definite, nitrification; about 12 per cent. of its N was nitrified after 240 days, in which time 15 per cent. of its C also disappeared, so that its C:N ratio remained nearly the same. The Edelmist humus was more resistant; not until after 7–8 months was there a definite, although slight nitrification. The manure humus with the wider C:N ratio was still more slowly nitrified, but its carbon compounds were attacked to a larger extent, so that this material tended to adjust itself to the same C:N ratio as the others. Microscopic examinations showed the presence of a large number of small rods and cocci, especially in the sand + manure humus. We have only a few records in the literature, of laboratory experiments on decomposition of humus. Rimbach[62] added Ca-humate with 4 per cent. N to sand and found 5·9 per cent. of its N nitrified after 2 months. Nikitinsky[51] found humic acid decomposed both in the presence and absence of living micro-organisms, but more rapidly in the former case. Fraps[18] added humic acid to soil and found about 3 per cent. of its C given off as CO_2 in 6 weeks under conditions where cotton-seed meal and manure lost 62 and 30 per cent. of their C. Löhnis and Green[41] found that humus from peat was very slowly nitrified in the soil, humus from manure more rapidly, and humus from plant materials (probably containing some easily decomposable protein material) still more rapidly;

the rate of nitrification was, to a certain degree, proportional to the percentage of N. Liesche(38) prepared humus from Edelmist and found that 2·5–3·4 per cent. of its N was nitrified and 1·5–2·1 per cent. of its C disappeared in soil or sand after 4 weeks. The material was not boiled in acid, so that some easily decomposable N compounds may have been present, which may account for the discrepancy with the present results. The only observation of actual decomposition of humus by bacteria is due to Winogradsky(86), who found colonies of small rod-shaped bacteria developing on silica gel plates with Ca-humate. The colonies very slowly formed transparent haloes.

All these observations show conclusively that the nitrogen in the humus fraction of the manure is present in a state where it is very slowly mobilised by the soil micro-organisms, a fact which was suspected almost 60 years ago by Déhérain(11), whose words can still be fully endorsed to-day: "Quant à la matière noire, dernier terme de la fermentation du fumier, il est probable qu'elle résiste plus longtemps, que ce n'est que lentement qu'elle se brûle, mais qu'elle fournit encore des nitrates comme dernier produit d'oxydation. Elle constitue sans doute la plus grande partie de cette *vieille force*, qui s'accumule dans les terres bien fumées."

(8)   *Origin of the humus fraction.*

The question now arises: what is the origin of this humus fraction? It has sometimes been suggested that the process of condensation between sugars and amino acids discovered by Maillard(45) might be responsible for the formation of humic matter both in soil and manure. Against this Waksman(80) and Liesche(38) have pointed out that sugars and amino acids are never found in the soil except in mere traces and, moreover, du Toit(74) and Marshall(46) found that the product of this reaction has physico-chemical properties which are quite different from those of soil humic acid. In manure it is conceivable that this process might take place, especially in Edelmist, where the temperature for a long time remains at 50–60° C. Löhnis(44) even speaks of this process as *the* mode of formation of humus in Edelmist. The weak point of the theory is that the process seems to have been studied only in concentrated solutions of sugars and amino acids. A small experiment on the importance of the concentration was carried out.

1.   50 c.c. of a sterile solution of 2 per cent. dextrose and 0·5 per cent. glycine (solutions sterilised apart and mixed) were kept in a cotton-plugged flask at 54° C. After 5 days the fluid had a slight yellow tinge; after 10 days it was straw coloured; and after 21 days, when its original

volume through evaporation had been reduced to 10 c.c., it was deep orange. No precipitate was obtained on acidification with hydrochloric acid.

2. 50 c.c. of a sterile solution of 4 per cent. dextrose and 1 per cent. glycine kept at 54° C. After 2 days a faint yellow tinge appeared and became gradually more intense, until after 24 days the solution was coffee-brown. No precipitate on acidification. The solution had evaporated down to 10 c.c.

3. Same solution kept at 37° C.: straw yellow after 24 days.

4. 50 c.c. of a sterile solution of 8 per cent. dextrose and 2 per cent. glycine at 54° C. Solution became orange after 5 days, and after 14 days, when it had evaporated down to 15 c.c., it was intensely brown, and transparent when diluted. On acidification with HCl it yielded 0·050 gm. of an amorphous, dark brown precipitate containing 8 per cent. N.

This simple experiment shows that there is indeed a formation of coloured matter, but even in solution of 4 per cent. dextrose and 1 per cent. glycine no production of any substance precipitated by acid, which might be accepted as α-humus or humic acid, after more than 3 weeks' incubation at 54° C. (which is about the temperature of Edelmist after the first days of aerobic fermentation), even when we allow for the increase in concentration due to evaporation. And such a concentration of sugars and amino acids is hardly imaginable in a medium where living organisms are constantly present (the Edelmist is indeed poor in bacteria, but never sterile, and thermophilous organisms would probably flourish to an enormous extent if such concentrations of excellent nutrients like sugars and amino acids arose). It is thus evidently not here that we have to look for the source of nitrogenous humus. But the studies of recent years have indicated that lignins are the source of the bulk of the soil humus, although the fraction containing N must have another origin. Straw, which is present in abundance in the manure, contains about 20 per cent. of lignin, which thus is most likely to be found in the humus of the manure. The determinations of methoxyl in the humus as well as the carbon determinations also point definitely in this direction. But what is the source of the comparatively high N content of the humus? It was suggested in 1888 by Dehérain [12] that the humus of manure may be a mixture of lignins and proteins built up by bacteria, and it is most likely that some protein material will be extracted and precipitated along with the lignin. We cannot, however, be dealing with an ordinary protein, mechanically mixed with the lignin, since normal proteins are easily attacked by bacteria (Balks [4]), and in such a case we should

have expected that the decomposition experiment with humus would have shown at first a rapid ammonia or nitrate production, later perhaps followed by a consumption of these compounds, when the lignins begin to undergo decomposition. Hobson (25) suggested that soil humic acid may be regarded as an adsorption compound between lignins and certain proteins, which latter are protected through the adsorption against bacterial attack, and he demonstrated that a composite of lignin and albumen, obtained by mixing alkali solutions of the two compounds and precipitating them together by means of acid, shows an amino N distribution almost identical to that of soil humic acid, when analysed by the van Slyke method. Some experiments were carried out in order to test this point. Mixtures of lignin and protein were prepared by mixing NaOH solutions of carbohydrate-free lignin[1] and casein and precipitating the mixture by means of HCl. Three samples containing 3·9–4·1 per cent. N were prepared, and, when added to mineral nutrient solution inoculated with soil suspension and kept at 25° C., only a trace of ammonia formation took place after 3 weeks, whereas in a control solution containing a corresponding amount of N as casein, 75–80 per cent. of this was ammonified. However, in a similar experiment carried out in soil, this resistance to microbial attack was not nearly so marked, and only an insignificant part of the added N was recovered in the humus after 45 days. It does not seem that lignin will form such resistant adsorption compounds with every protein, but the suggestion is interesting and should be tested further. A third possible source of nitrogenous humus is the microbial protoplasm, which may contain compounds that are in themselves very resistant or perhaps able to form resistant compounds with the lignin.

DISCUSSION.

All these experiments show us that the N of farmyard manure is nitrified rather slowly and never completely—a result which is in agreement with our experience from field experiments. The degree of nitrification does not depend so much on whether the N is present as ammonia or organic compounds, as on the C:N ratio of the manure and the content of relatively undecomposable humus-like compounds. When added to the soil, the manure introduces a large quantity of energy material, which enables the micro-organisms of the soil to develop abundantly for a shorter or longer period, especially when fresh straw is present in the manure. This multiplication of micro-organisms entails the locking up

[1] Kindly supplied by Mr G. V. Jacks, B.A., B.Sc., Chemical Department.

of considerable amounts of N in protoplasm, and the nitrification is therefore retarded, until the numbers of micro-organisms have again decreased. The rise in nitrate production, which sets in at this period, corresponds apparently to the decomposition of the dead bacterial bodies. The nitrification does not become complete within 16 months; but its rate gradually diminishes, and a considerable residue of nitrogenous compounds resists decomposition. A part of this is evidently the humus fraction of the manure, but this does not account for all of it. An explanation for the origin of these residues will be sought in a following section of this work.

The remarkable losses of N in the manured soils are difficult to account for. The only microbial process which is known with certainty to give rise to a development of elementary N is denitrification. That this may have occurred in the Woburn soil is possible, though not certain, and it is unlikely that this should be responsible for the heavy losses of N repeatedly observed in manured, tilled soil. These losses seem connected with good aeration, which is not favourable to denitrification (although not at all detrimental to the existence of the denitrifying bacteria themselves). In accordance herewith, Russell and Richards(65) found no loss of N from manure decomposing under perfectly aerobic or perfectly anaerobic conditions, whereas a considerable loss occurred under semi-aerobic conditions. These latter would be more likely to exist in an untilled than in a tilled soil, from which the losses of N are heavy (Shutt(72)). Apart from denitrification, the possibilities of losses of elementary N through microbial processes are only few and little known. The *B. azotofluorescens*, claimed by Kaserer(32) to be able to oxidise ammonia to water and free N, has not been found by other investigators. Schittenhelm and Schröter's(70) observation that N is evolved from nucleic acid when decomposed by *B. coli*, was shown by Oppenheimer(55) to depend on analytical errors. Pfeiffer and Lemmerman(57) found that soil with addition of manure and nitrate lost N to an extent which could hardly be ascribed to denitrification alone, and Sabachnikoff(67) came to a similar conclusion. Recently Lemoigne and Dopter(37) claim to have isolated a number of bacteria and actinomycetes, which cause a loss of considerable amounts of N from pure cultures under conditions where precautions are taken against the evaporation of ammonia. Their very brief paper unfortunately does not give any idea either of the identity of the organisms or the biochemical nature of the processes which cause the losses of N.

There is thus a certain discrepancy between the field and the labo-

ratory experiment. In the former case losses of N seem to be the rule, but in the experiments described here there was a significant loss of N only in the limed Woburn soil, although the additions of manure were in all cases enormous. The experiments in Hoosfield soil and pure sand show that the decomposition of manure can go very far without a significant loss of N occurring, as was also found by Joshi(31). The possibility remains that we are here neglecting a physico-chemical factor, namely the influence of light, which is of course far stronger in the field than in the laboratory experiments. Berthelot(7) observed that humic acid, when exposed to light, absorbs O_2 and produces CO_2; this observation was later definitely confirmed by Nikitinsky(51). It seems not impossible that such a purely chemical decomposition of humus might give rise to an evolution of free N.

SUMMARY.

Decomposition experiments were carried out in the laboratory with different kinds of farmyard manure in various soils (sand and clay, acid and neutral). In neutral or slightly acid soil there was a very strong multiplication of bacteria and, to a smaller extent, of actinomycetes immediately after the addition of manure. This increase, which was especially marked when fresh straw was present in the manure, was sooner or later followed by a rather sudden decrease, which caused the numbers of bacteria gradually to approach those in the control soils. The actinomycetes were generally more abundant in the later stages of the process. This suggests that they may be especially active in the decomposition of the more resistant residues. The "numbers" of fungi were not affected by the addition of manure alone (except to a slight degree in strongly acid soil, where the bacteria seemed inactive), but the presence of fresh straw caused them to become active, especially in strongly acid soil, where their "numbers" remained at a very high level for a long time, this abundance of fungi consisting of both mycelium and spores.

The nitrification of the manure N became active at the period when the bacterial, or fungal, numbers were again decreasing. The statement that the organic N of the manure does not undergo any nitrification during the first year could not be confirmed, but the nitrification of the organic N was found to be incomplete, since it became gradually slower and seemed to tend to come to a standstill. The α-humus fraction of the manure contained a considerable amount of N in a very inert form.

This fraction, which contained 18–25 per cent. of the total organic N of the manure, did not undergo any significant decomposition in the soil during 6–12 months, after which time it could be recovered in the α-humus fraction of the soil, often together with some extra humus, which seemed to have been synthesised during the process of decomposition. The humus fraction of the manure consisted largely of lignin, probably combined with some proteid material.

The C:N ratio of the manure exerted a great influence upon the degree and the rapidity of the nitrification of the manure. The so-called Edelmist showed a stronger nitrification than ordinary farmyard manure, but this seemed merely dependent on its narrower C:N ratio; its N compounds did not seem more easily decomposable than those of ordinary manure.

Control experiments with dried manure in sand gave similar results, but the decomposition seemed to proceed somewhat more rapidly than in the soils. The manures tended to adjust themselves to a C:N ratio of 11–12:1, and no loss of N took place.

In the soils there was in some instances a significant loss of total N, which might be due to denitrification.

The comparatively low fertilising value of the organic N of the manure seems to depend on the following phenomena: the organic matter of the farmyard manure is a mixture of compounds of a fairly wide C:N ratio. When this is added to the soil, the various compounds are attacked by the bacteria, actinomycetes and fungi, and a part of the available N of the manure is used up as nitrogenous food by the micro-organisms. When the supply of readily available energy material is exhausted, the bacterial numbers drop, and a production of mineral N begins. This production diminishes gradually without, in any case, reaching the total amount of N in the manure. In this respect the farmyard manure resembles other organic manures, which generally yield only a fraction of their N as nitrate, but here the phenomena are somewhat more complicated owing to the presence of the resistant humus fraction in the manure.

78　*Microbiology of Farmyard Manure Decomposition*

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THE MICROBIOLOGY OF FARMYARD MANURE DECOMPOSITION IN SOIL.

II. DECOMPOSITION OF CELLULOSE.

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In a previous paper [12] it was pointed out that the marked increase in bacterial numbers immediately after the addition of manure to the soil is inversely correlated with the production of ammonia and nitrate from the manure, apparently because the bacteria derive the energy for their increase mainly from non-nitrogenous compounds, and assimilate the available nitrogen which is not released again until after the death and subsequent decomposition of the bacterial cells. Cellulose and lignins represent the main groups of energy material in well decomposed manure while fresh manure and straw also contain pentosans in considerable amount. It is presumably from the cellulose and pentosans that the organisms derive the energy for their initial increase, since numerous studies have agreed in showing that the lignins are only very slowly decomposed and contribute largely to the formation of humus in soil and manure. The present contribution deals with the decomposition of the cellulose.

The micro-organisms capable of decomposing celluloses are of widely different groups:

1. *Aerobic mesophilic bacteria* (v. Iterson [11], McBeth and Scales [18], Hutchinson and Clayton [10], Gray and Chalmers [8], Winogradsky [32], Dubos ( ), Kalninš [13]).

2. *Anaerobic mesophilic bacteria* (Omeliansky [19], Khouvine [14]).

3. *Thermophilic bacteria* (Pringsheim [21], Kroulik [16]).

4. *Actinomycetes* (Krainsky [15]).

5. *Filamentous fungi* (v. Iterson [11], Daszewska [3], McBeth and Scales [18], Otto [20], Waksman and Heukelekian [9, 28], Rege [22]).

6. *Higher fungi* (Tubeuf [26], Malenkovic [17], Wehmer [31], Rege [22]).

Of these groups the anaerobic bacteria are hardly active in normal

soils (Waksman and Skinner(29), Dubos(4)), although undoubtedly of importance in the manure heap. The same is probably true of the thermophilic bacteria. The actinomycetes mostly exert only a slight influence upon the cellulose and seem of little importance except in dry soils (Dubos(4)). The higher fungi are extremely important agencies in decaying wood (see Thaysen and Bunker(24)) and consequently in forest soil, but their importance in field soil is unknown, although it may be considerable. We are thus left with the groups 1 and 5, viz. the aerobic mesophilic bacteria and the filamentous fungi, which both become active when cellulosic material is added to the soil (Waksman and Heukelekian(28), Waksman and Skinner(29), Dubos(4), Winogradsky(32)). The importance of these organisms in the decomposition of farmyard manure in the soil has not received much attention, and most of the work has been carried out with pure cellulose, a compound occurring in nature in far smaller amounts than the lignified cellulose of straw and wood, which is much more slowly decomposed (Barthel and Bengtsson(1), Rege(22)). In the present contribution the following questions have been considered:

1. What types of cellulose-decomposing micro-organisms are active, when farmyard manure is decomposed in the soil?

2. How large amounts of nitrogen are assimilated by the various organisms in proportion to the amounts of decomposed cellulose?

3. Are humus-like nitrogenous compounds formed by the cellulose-decomposing micro-organisms?

I. TYPES OF CELLULOSE-DECOMPOSING ORGANISMS IN VARIOUS SOILS WITH ADDITION OF MANURE AND STRAW.

In three of the series of manure decomposition experiments described in the previous paper(12) determinations of the relative abundance of cellulose-decomposing bacteria were carried out by means of the dilution method used by Dubos(4): test-tubes with strips of filter-paper (Whatman No. 41) half immersed in a mineral nutrient solution[1] were inoculated with 1 c.c. portions of soil suspension of increasing dilution, and incubated at 25° C. for 3–4 weeks. In cases where stimulation of the soil fungi (determined by the plate method) had taken place, the more conspicuous forms were isolated and tested for their ability to grow on cellulose in the form of filter-paper.

[1] NaNO$_3$, 2·0 gm.; K$_2$HPO$_4$, 0·5 gm.; MgSO$_4$, 0·2 gm.; NaCl, 0·2 gm.; FeCl$_3$, trace; distilled water, 1000 c.c.

The following results were obtained:

Neutral Park plot soil, pH 7·0, *with addition of manure and oat straw.*

(*a*) Results of the dilution method; tubes incubated 100 days at room temperature:

Control soil. Four tubes inoculated with soil suspension diluted 1:200 showed the paper in two tubes broken at level of solution and in two not attacked.

Soil + manure. Four tubes inoculated with soil suspension diluted 1:1000 showed the paper in all tubes broken at level of solution, which was colourless to pale yellow. Four tubes inoculated with soil suspension diluted 1:10,000 showed the paper in three tubes broken, and in one not attacked.

Soil + manure + straw. Four tubes inoculated with soil suspensions diluted 1:10,000 showed the paper in three tubes broken at level of solution, and in one yellow zone. Five tubes inoculated with soil suspension diluted 1:100,000 showed the paper in three tubes broken at level of solution, and in two not attacked.

The microscopic picture of the paper from the tubes showing an attack was the same in all cases: an abundance of small, actively motile, *Vibrio*-like organisms were present. Pure cultures of these could be obtained by streaking the liquid on an agar medium with mineral nutrients and finely divided hydrocellulose prepared by the method of Scales(23). When incubated for 6-8 days at 25° C., small colourless colonies appeared along the streaks, surrounded by very distinct clear haloes. When transfers were made to filter-paper, decomposition of this would set in after a few days' incubation at 25° C. One strain, isolated from soil + manure and termed *Vibrio* B.M., was kept for further study. Young cultures (2 days) on filter-paper: slightly curved, slender rods, 1·7-2·5 × 0·5-0·6 μ, actively motile by means of one polar flagellum. In older cultures short, almost coccoid, forms are seen. Gram-negative. Endospores are not formed. Nitrate is not reduced. Starch is hydrolysed. Agar is not liquefied. Strictly aerobic. Growth is better at 25° C. than at 20° C.; no growth at 37° C. On filter-paper strip in mineral nutrient solution the attack is already visible after 2 days, and after 3 days the paper breaks into two at the level of solution, where a narrow zone of the paper is transformed into a soft pulp. The solution becomes faintly turbid, and a faint lemon-yellow pigment is formed in the paper. The

mode of attack is much the same as that of *Vibrio* (*Microspira*) *agar-liquefaciens* (Gray and Chalmers(8)), to which the organism appears closely related.

(*b*) *Activity of fungi.* As shown in the previous paper, the manure caused no increase of fungi in this soil, but the addition of straw gave rise to an enormous development of fungi, particularly of *Cephalosporium* sp., which, however, did not decompose cellulose in pure culture. Special fungi, to which the plate method is not readily applicable, seemed, however, to be active here. A plating on cellulose agar from soil + manure yielded a peculiar fungus—apparently a *Botryosporium*, forming numerous clusters of black chlamydospores which decomposed cellulose very actively in pure culture. The same fungus could be obtained by planting the half-decomposed bits of straw from the soil + manure + straw directly into plates of cellulose agar. The fungus appeared remarkably sensitive to acid reaction; this was probably the reason why it was not obtained by the plate method.

SERIES 1 B.

Acid Park plot soil, pH 3·8, with addition of manure and straw.

(*a*) Results of the dilution method; tubes incubated for 100 days at room temperature:

Control soil. Two tubes inoculated with soil suspension diluted 1:100; one tube showed a doubtful attack and one a development of fungi.

Soil + manure. Two tubes inoculated with soil suspension diluted 1:100 showed in one tube a doubtful attack, and in one a development of *Spirochaeta cytophaga*. Two tubes inoculated with soil suspension diluted 1:5000 both remained sterile.

Soil + manure + straw. Two tubes inoculated with soil suspension diluted 1:200 showed in one tube a development of fungi and in one of *Spirochaeta cytophaga*. Two tubes inoculated with soil suspension diluted 1:5000 showed one tube with a development of fungi and one sterile.

There is thus no significant development of cellulose-decomposing bacteria in this very acid soil. The presence of *Spirochaeta cytophaga*, which occasionally develops in low dilutions, may be due to its having been introduced with the manure.

(*b*) *Activity of fungi.* As shown before, the soil with manure and straw showed an enormous development of fungi, especially *Trichoderma* sp. and *Amblyosporium* sp. (?), forms which both grew well on cellulose in pure culture.

SERIES 2A.

Faintly acid sandy soil from Woburn (pH 5·5–5·8), with addition of manure, ammonium sulphate, and straw.

(a) Results of the dilution method after 45 days' incubation at room temperature:

Soil	Dilution	Tube no.	Development of micro-organisms
Control	1:200	a b c	Sterile
Soil + manure	1:250	a	Paper broken, colourless
		b	„
		c	Sterile
	1:2500	a	Paper broken, colourless
		b	„
		c	Yellow spots, paper not attacked
Soil + manure + $(NH_4)_2SO_4$	1:250	a b c	*Spirochaeta cytophaga*
	1:2500	a b c	Sterile
Soil + manure + $(NH_4)_2SO_4$ + straw	1:5000	a b c	*Spirochaeta cytophaga*

Repeated examination after 90 days:

Soil	Dilution	Tube no.	Development
Control, pH 5·7	1:200	a	Paper broken, colourless
		b	Fungi
		c	„
Soil + manure, pH 5·8	1:25,000	a b c d	Paper broken, colourless
Soil + manure + $(NH_4)_2SO_4$, pH 5·5	1:2500	a	Fungi
		b	„
		c	Sterile
		d	„
Soil + manure + $(NH_4)_2SO_4$ + straw, pH 5·7	1:2500	a	Fungi
		b	„
		c	Sterile
		d	„

Spirochaeta cytophaga was active to some extent in the manured soils during the first 45 days, especially in the soil with manure and straw, and organisms of the *Vibrio*-type were active in the least acid soil (soil + manure), where they continued to flourish after 90 days. In the soils of pH 5·5–5·7 no activity of cellulose-decomposing bacteria was apparent after 90 days.

(*b*) *Activity of fungi.* The plate counts showed a considerable development of fungi in soil + manure + ammonium sulphate + straw, especially of a *Monosporium* sp., which made a good growth on filter-paper in pure culture.

SERIES 2B.

Same soils and additions as Series 2A, +1 per cent. CaCO$_3$, pH 6·5–6·7.

(*a*) Results of the dilution-method after 45 days:

Soil	Dilution	Tube no.	Development of micro-organisms
Control	1:200	a	*Spirochaeta cytophaga*
		b	,,
		c	Fungi
	1:2000	a	*Spirochaeta cytophaga*
		b	Sterile
		c	,,
Soil + manure	1:25,000	a b c	Paper broken, colourless
	1:100,000	a	Paper broken, colourless
		b	,,
		c	Sterile
Soil + manure + (NH$_4$)$_2$SO$_4$	1:25,000	a b c	Sterile
Soil + manure + (NH$_4$)$_2$SO$_4$ + straw	1:25,000	a b c	Paper broken, colourless
	1:100,000	a	Paper broken, colourless
		b	,,
		c	,,
		d	Sterile

Examination after 90 days gave similar results.

There was thus an abundant development of bacteria of the *Vibrio*-type in these nearly neutral soils, except in the soil with ammonium sulphate. Microscopically the cultures appeared like those of Series A1. A strain termed *Vibrio* W.6 was isolated from soil + manure. It appeared much like *Vibrio* B.M., but did not form any yellow pigment and reduced nitrate to nitrite.

(*b*) *Activity of fungi.* The plate counts did not show any significant stimulation of the fungi in these soils.

SERIES 3.

Faintly acid clay from Hoosfield (pH 6·3), with addition of farmyard manure and Edelmist.

(a) Results of the dilution method after 30 days at room temperature:

Soil	Dilution	Tube no.	Development of micro-organisms
Control	1:200	a	*Spirochaeta cytophaga*
		b	,,
		c	Paper broken, colourless
		d	Sterile
Soil + farmyard manure	1:5000	a ⎫ b ⎬ c ⎭	Paper broken, colourless
	1:25,000	a ⎫ b ⎪ c ⎬ d ⎭	Paper broken, colourless
Soil + Edelmist	1:5000	a ⎫ b ⎬ c ⎭	*Spirochaeta cytophaga*
	1:25,000	a	Paper broken, colourless
		b	*Spirochaeta cytophaga*
		c	Sterile
		d	,,

There is here again a decided stimulation of the *Vibrio*-like organisms in soil + ordinary manure, and of *Spirochaeta cytophaga* in soil + Edelmist. An organism, termed *Vibrio* H.M., was isolated from soil + manure. It resembled *Vibrio* B.M., but it did not form any yellow pigment, and its cells were somewhat thinner ($0·3-0·4\,\mu$).

(b) *Activity of fungi.* No significant stimulation occurred in any of the soils.

All these experiments show a definite stimulation of cellulose-decomposing bacteria due to addition of manure and straw in faintly acid to neutral soils (pH 6·3–7·0). At pH 5·7–5·8 the multiplication of these organisms is far less pronounced, and at pH 5·5 it seems quite absent. *Spirochaeta cytophaga* has a tendency to develop at the faintly acid reactions (pH 5·8–6·3), whereas the vibrios are decidedly more abundant at reactions nearer to the point of neutrality.

In order to see whether similar results can be obtained with other soils, some control experiments were carried out by adding cellulose as filter-paper or ground wheat straw to soils of different reaction. This also gives us a control on the source of errors possibly due to the introduction of large numbers of living cellulose-decomposing bacteria in fresh farmyard manure.

First control experiment.

Soils of pH values from 4·3 to 7·1 received 1 per cent. filter-paper or wheat straw, distilled water to approximately 70 per cent. of the water-holding capacity, and 21·3 mg. of N as ammonium phosphate per 100 gm. of dry soil. Samples of 200 gm. were kept in a moist condition at room temperature.

The following soils were used:

(1)	Clay soil from Cheshire	pH 4·3
(2)	Sandy soil from Tunstall	pH 4·9
(3)	Mixture of soils 1 and 4	pH 5·9
(4)	Clay soil from Hoosfield	pH 6·2
(5)	Garden soil, heavy loam	pH 7·1

The dilution method gave the following results:

1 (*a*). *Cheshire soil pH* 4·3 + *filter-paper.* No development of cellulose decomposing bacteria after 20 or 45 days.

1 (*b*). *Do.* + *straw.* No cellulose decomposing bacteria after 20 or 45 days.

2 (*a*). *Tunstall soil, pH* 4·9 + *filter-paper.* No cellulose decomposing bacteria after 20 or 45 days.

2 (*b*). *Do.* + *straw.* No cellulose decomposing bacteria after 20 or 45 days.

3 (*a*). *Mixed soil, pH* 5·9 + *filter-paper.* No cellulose decomposing bacteria after 20 or 45 days.

3 (*b*). *Do.* + *straw.* No cellulose decomposing bacteria after 20 days. After 45 days three tubes inoculated with soil suspension diluted 1:1000 showed two tubes with development of *Spirochaeta cytophaga.*

4 (*a*). *Hoosfield soil, pH* 6·2 + *filter-paper.* After 20 days:

Dilution	Tube no.	Development of micro-organisms
1:10,000	*a* *b*	Paper broken, yellow
1:100,000	*a*	Paper broken, yellow
	b	Sterile
1:1,000,000	*a* *b* *c*	Sterile

Do. After 45 days:

1:10,000	*a* *b* *c*	*Spirochaeta cytophaga*
1:100,000	*a*	*Spirochaeta cytophaga*
	b	Paper broken, colourless
	c	Sterile

4 (*b*). *Same soil + straw.* After 20 days:

Dilution	Tube no.	Development of micro-organisms
1:10,000	*a* *b*	Paper broken, colourless
1:100,000	*a* *b* *c*	No attack

Do. After 45 days:

1:100,000	*a*	*Spirochaeta cytophaga*
	b	Sterile
	c	,,

5 (*a*). *Garden soil, pH* 7·1 *+ filter-paper.* After 20 days:

1:100,000	*a* *b* *c*	Paper broken, colourless
1:1,000,000	*a*	Paper broken, colourless
	b	No attack

5 (*b*). *Same soil + straw.* After 20 days:

1:100,000	*a*	Paper broken, colourless
	b	
	c	No attack ,,
	d	,,

Do. After 45 days:

1:100,000	*a*	*Spirochaeta cytophaga*
	b	No attack
	c	,,

This experiment thus gave the same general results as the main experiment: at *p*H 5·9 there was only a very slight development of *Spirochaeta cytophaga*, at *p*H 6·2 this organism became more active, and at *p*H 7·1 the vibrios prevailed. At lower *p*H values only the fungi appeared to be active. Plate counts of the fungi showed an abundance of Penicillia, Trichodermae and *Monosporium* in the first three soils, besides *Mucor Ramannianus* in straw treated soils. In the last two soils, especially with addition of straw, the development of the fungi was less abundant, and the most prevalent forms were *Monosporium* sp., *Mycogone nigra*, *Stachybotrys* sp., and an organism resembling *Coccospora agricola* described by Goddard[7].

Second control experiment.

Soil	*p*H	Addition
Clay soil, Cheshire	4·3	
Sand soil, Woburn	5·7	1 gm. filter-paper + 21 mg. N as
Garden soil, Rothamsted	7·1	NaNO$_3$ per 100 gm. soil

Results of the dilution method after 45 days' incubation at 25° C.:

Soil	Dilution	Tube no.	Development of micro-organisms
Cheshire soil	1:100	a b c	Sterile
Woburn soil	1:100,000	a b c d	*Spirochaeta cytophaga*
Garden soil	1:10,000,000	a	Paper broken, colourless
		b	,,
		c	,,
		d	*Spirochaeta cytophaga*

Here again *Spirochaeta cytophaga* developed in the faintly acid Woburn soil, the reaction of which changed during the experiment to pH 6·6, and the vibrios preponderated in the neutral soil. The microscopical appearance of the cultures where the paper was broken at the level of the solution was the same in both the control experiments and in the main experiments: numerous small vibrios, of which one organism, termed *Vibrio* G.St., was isolated from garden soil + straw.

Table I. *Growth of cellulose-decomposing bacteria at varying hydrogen ion concentration.*

Organism	Growth at pH*							
	7·6	7·1	6·8	6·4	6·0	5·6	5·2	4·6
Vibrio prima (Kalninš)	+ (2)	+ (2)	+ (4)	+ (6)	–	–	–	–
Vibrio napi (Kalninš)	+ (3)	+ (3)	+ (4)	+ (5)	–	–	–	–
Vibrio pericoma (Kalninš)	+ (10)	+ (10)	+ (20)	–	–	–	–	–
Vibrio bulbosa (Kalninš)	+ (10)	+ (10)	+ (20)	–	–	–	–	–
Vibrio agarliquefaciens	+ (4)	+ (5)	+ (7)	+ (12)	–	–	–	–
Vibrio B.M.	+ (3)	+ (3)	+ (5)	+ (5)	–	–	–	–
Vibrio W. 6	+ (3)	+ (3)	+ (3)	+ (4)	–	–	–	–
Vibrio H.M.	+ (4)	+ (4)	+ (4)	+ (5)	–	–	–	–
Vibrio G.St.	+ (4)	–	+ (8)	+ (10)	–	–	–	–
Vibrio from tap-water	+ (2)	+ (3)	+ (3)	+ (3)	+ (6)	+ (20)	+ (20)	–
Spirochaeta cytophaga, impure from Woburn soil	+	+	+	+	+	+	–	–
Spirochaeta cytophaga, pure	+	+	+	+	+	–	–	–

* Figures in brackets indicate number of days which elapsed before paper was broken. Two or three parallel cultures, incubated at 25° C. for 30 days.

A third control experiment was carried out with some soils from abroad: viz. three Gold Coast soils, of pH 4·8, 5·2 and 5·9; two Tchernozem soils from Bessarabia, of pH 6·6 and 7·4; and an alkali soil from Sudan, of pH 9·2. Addition of cellulose and ammonium sulphate gave rise to an abundant development of Penicillia and Trichodermae in the first three soils. In both the Tchernozem soils there was a strong development of *Mycogone nigra*, in that with a reaction of pH 7·4 there was also a growth of cellulose-decomposing vibrios, this being the only soil of the series in which cellulose decomposing bacteria appeared active. In the alkali soil there was a fairly strong development of *Stachybotrys* sp.

This close correlation between soil reaction and types of cellulose-decomposing bacteria made it seem desirable to test the ability of the various bacteria to grow at different degrees of acidity. The vibrios mentioned above and a not quite pure culture of *Spirochaete cytophaga* from Woburn soil were grown on filter-paper strips in test-tubes with the mineral nutrient solution mentioned above, p. 82, in which the 0·05 per cent. K_2HPO_4 was replaced by 0·2 per cent. of a mixture of varying proportions of KH_2PO_4 and K_2HPO_4:

Buffer mixture	pH after sterilisation
0·2 % KH_2PO_4	4·6
0·008 % K_2HPO_4 + 0·192 % KH_2PO_4	5·2
0·02 % K_2HPO_4 + 0·18 % KH_2PO_4	5·6
0·04 % K_2HPO_4 + 0·16 % KH_2PO_4	6·0
0·1 % K_2HPO_4 + 0·1 % KH_2PO_4	6·4
0·12 % K_2HPO_4 + 0·08 % KH_2PO_4	6·8
0·16 % K_2HPO_4 + 0·04 % KH_2PO_4	7·1
0·2 % K_2HPO_4	7·6

For comparison, the following authentic cultures of cellulose decomposing bacteria were included in the experiment: *Spirochaeta cytophaga*, a pure culture kept for several years in the laboratory. *Vibrio agarliquefaciens*, freshly isolated from garden soil. *Vibrio prima, napi, bulbosa* and *pericoma*[13] received from Dr A. Kalniņš, University of Riga, Latvia. In addition, a vibrio-like organism, found as accidental infection in tubes with filter-paper and probably coming from tap-water, was included. The results are shown in Table I.

The experiment shows plainly that the vibrios from the soil as well as Kalniņš' organisms are very sensitive to acidity, since they fail to develop in the pH interval 6·0–6·4. *Spirochaeta cytophaga*, especially the freshly obtained, impure culture, is a little more resistant. These facts agree perfectly with the relative prevalence of these organisms in the soils of different reaction, as shown by the dilution method. While these experiments were in progress there appeared two papers by

Dubos (4, 5), whose results agree with the present ones. When filter-paper or straw was added to soil of different reaction, a multiplication of cellulose-decomposing bacteria took place only at pH values not lower than 6·0, and most strongly at pH 7·0–8·0. Two cellulose-decomposing vibrios failed in pure culture to develop in the pH interval 6·0–6·5; of two other organisms one behaved similarly, and one was still able to grow at pH 6·0. *Spirochaeta cytophaga* would, when low concentrations of N were used, grow at pH 5·2–5·7. The *Vibrio* from tap-water studied here is considerably more resistant to acidity, but it does not seem active in the soil, since in that case it would probably have been noticed in the dilution experiments, being easily recognised by its formation of a rust-brown pigment in the paper.

II. ACTION OF VARIOUS ORGANISMS ON CELLULOSE IN PURE CULTURE.

The comparative cellulose-decomposing power of the various organisms obtained from the above-mentioned experiments was tested by growing them on pure quartz sand with addition of some cellulosic material and mineral nutrient solution. 150 gm. portions of sand were placed in round 300 c.c. flasks stoppered with tight-fitting perforated rubber stoppers, in which the holes were filled with cotton-wool. At the end of the period of incubation the sand was dried, and cellulose was determined. In the case of pure cellulose this was done by means of the modification of Charpentier's (2) method used by Gray and Chalmers (8): 20 gm. of sand were shaken for 1 hour with 100 c.c. of Schweitzer's solution, the mixture was allowed to settle overnight in a glass cylinder, 50 c.c. were pipetted off, and cellulose was precipitated by addition of an excess of 10 per cent. HCl, filtered off on a dried and weighed filter, washed, dried for 20–24 hours at 35° C., and weighed. For determinations of cellulose in straw, the method of Waksman and Tenney (30) was used: 20 gm. of dried sand were heated in the autoclave for 30 min. with 50 c.c. of a 5 per cent. NaOH solution, the liquid was poured off, extraction repeated, and the residue filtered off and boiled for 30 min. in an approximately 2 per cent. solution of sulphuric acid. After filtering and washing, the sand was dried and extracted with Schweitzer's reagent as before. The figures given in Tables II–V represent the means of duplicate determinations.

FIRST EXPERIMENT.

Sand + 2 *per cent. ground oat straw* + 1 *per cent.* $CaCO_3$ + 15 *per cent. nutrient solution* (($NH_4)_2SO_4$ 1 *per cent.*, KH_2PO_4 0·2 *per cent.*, $MgSO_4$ 0·1 *per cent.*, *NaCl* 0·1 *per cent.*).

The following organisms were studied: *Trichoderma* sp. from acid Park plot soil + manure + straw. *Aspergillus fumigatus* from fermenting straw. *Botryosporium* sp. from neutral Park plot soil + manure. *Vibrio* B.M. from the same soil. The first two fungi were also tested in culture without $CaCO_3$. Cultures were incubated for 45 days at 25° C., except the cultures of *Aspergillus fumigatus*, which were incubated at 38° C., the optimal temperature for this organism. The results are shown in Table II.

Table II. *Decomposition of cellulose of oat straw in sand culture.*

Culture	% of straw-cellulose decomposed
Trichoderma sp.	62·1
Do. + 1·0 % $CaCO_3$	10·4
Aspergillus fumigatus	75·9
Do. + 1·0 % $CaCO_3$	79·3
Botryosporium (2) sp. + 1·0 % $CaCO_3$	100·0
Vibrio B.M. + 1·0 % $CaCO_3$	63·8
Control	—
Do. + 1·0 % $CaCO_3$	—

The organisms were all capable of decomposing the cellulose of untreated straw, and the *Vibrio* was as active as *Trichoderma* sp. The activity of the latter organism was strongly reduced by the addition of lime (which agrees with the marked prevalence of this organism during cellulose decomposition in acid soil), whereas *Aspergillus fumigatus* was not affected by the change in reaction. The *Botryosporium* displayed a very strong activity.

SECOND EXPERIMENT.

Sand + 1 *per cent. finely cut filter-paper* + 15 *per cent. nutrient solution.* (*NaNO₃* 0·3 *per cent.*, K_2HPO_4 0·1 *per cent.*, $MgSO_4$ 0·05 *per cent.*, *NaCl* 0·05 *per cent.*).

Pure cultures of the following organisms were tested—all the vibrios isolated in the previous experiment, *Vibrio napi* and *prima* from Dr Kalnins, a pure culture of *Spirochaeta cytophaga*, and the following fungi: *Trichoderma* sp., *Botryosporium* sp., *Monosporium* sp. from Woburn soil, *Penicillium* sp. from Cheshire soil, *Mycogone nigra, Coccospora agricola* (?) and *Stachybotrys* sp. from garden soil. Cultures were incubated at 25° C. for 30 days. The results are found in Table III.

Table III. *Decomposition of cellulose as filter-paper in sand + neutral mineral salt solution, by bacteria and fungi.*

Culture	% of added cellulose decomposed	NO_3-N mg. per 100 gm. of sand	N resorbed (mg.)	Ratio cellulose decomposed: N resorbed
Vibrio napi (Kalnins)	34·7	0·0	11·4	30:1
Vibrio prima (Kalnins)	40·0	0·0	11·4	33:1
Vibrio B.M.	34·7	0·0	11·4	30:1
Vibrio H.M.	51·6	0·0	11·4	43:1
Vibrio W. 6	35·8	0·0	11·4	30:1
Vibrio G.St.	33·7	0·0	11·4	27:1
Vibrio from water	60·0	0·0	11·4	50:1
Spirochaeta cytophaga	55·8	0·0	11·4	47:1
Trichoderma sp.	0	10·9	(0)	—
Penicillium sp.	(0)	—	—	—
Coccospora sp. (2)	36·8	0·0	11·4	30:1
Monosporium sp.	30·3	0·0	11·4	25:1
Mycogone nigra	64·2	0·0	11·4	54:1
Botryosporium sp. (2)	57·9	0·0	11·4	48:1
Stachybotrys sp.	58·9	0·0	11·4	49:1
Control	—	11·4	—	—

Trichoderma and *Penicillium* were quite inactive here (the reaction was unfavourable, and nitrate is a poor source of N to them), but all the other organisms used up all the available N and decomposed rather unequal amounts of cellulose. Waksman and Heukelekian [9, 28] state that fungi decompose 30–35 units of cellulose for every unit of assimilated N. In the present experiment this was the case only with *Coccospora* sp. and *Monosporium* sp., whereas *Mycogone, Botryosporium* and *Stachybotrys* showed a considerably wider ratio, decomposing 48–54 units of cellulose for every unit of N. Waksman [27] mentions in a later communication that *Humicola* sp., an organism described by Traaen [25] and possibly identical with *Mycogone nigra*, shows a similar relationship. The nitrogen requirements of the bacteria were by no means smaller than those of the fungi, as was also suggested by Dubos [4]. Indeed, *Vibrio napi, prima*, B.M., W.6, and G.St. showed much the same cellulose : nitrogen ratio as found by Waksman and Heukelekian for the fungi, whereas the ratios for *Vibrio* H.M., *Spirochaeta cytophaga*, and the tap-water vibrio were wider, corresponding to *Spirochaeta cytophaga* and *Humicola* sp. according to Waksman [27]. It cannot, therefore, be a general rule that the bacteria consume less nitrogen than the fungi, as far as cellulose decomposition is concerned. In the case of *Spirochaeta cytophaga* Hutchinson and Clayton [10] found a cellulose : nitrogen ratio considerably narrower (27–30:1) than that observed here. The explanation may be that the amount of N was insufficient in the present experiments, so that some N from dead cells may have been utilised over again.

Also no direct determinations of N were made in Hutchinson and Clayton's experiments.

<div align="center">THIRD EXPERIMENT.</div>

Sand + 1 per cent. finely cut filter-paper + 15 per cent. mineral salt solution (($NH_4)_2SO_4$ *1 per cent.,* KH_2PO_4 *0·2 per cent.,* $MgSO_4$ *0·05 per cent.,* *NaCl 0·05 per cent.).*

Only the fungi were included in this experiment. Cultures were incubated for 30 days at 25° C. The results are shown in Table IV.

Table IV. *Decomposition of cellulose as filter-paper by fungi in sand with acid mineral salt solution.*

Culture	% of added cellulose decomposed	NH_4-N mg. per 100 gm. of sand	N resorbed (mg.)	Ratio cellulose decomposed: N resorbed
Trichoderma sp.	27·4	21·1	6·9	37:1
Penicillium sp.	20·0	23·6	4·4	44:1
Coccospora sp.	14·6	23·4	4·6	30:1
Monosporium sp.	26·5	22·1	5·9	44:1
Mycogone nigra	13·7	25·1	2·9	45:1
Botryosporium sp.	(0)	27·8	(0)	—
Control	—	28·0	—	—

There was less cellulose decomposition than in the previous experiment. *Trichoderma* and *Monosporium* were the most active organisms here. The activity of *Mycogone* and *Coccospora* was greatly diminished owing to the acidity reaction, and *Botryosporium* was rendered quite inactive. It is noteworthy that only *Trichoderma* and *Coccospora* showed approximately the cellulose:nitrogen ratio found by Waksman and Heukelekian for the fungi, whereas the three others showed a wider ratio, although there was here a considerable excess of NH_4-N and consequently no reason to suspect a repeated utilisation of N which was assimilated and again released, such as might have occurred in the previous experiment, where the whole supply of N was used up.

<div align="center">FOURTH EXPERIMENT.</div>

Sand + 1 per cent. crude[1] lignocellulose + 1 per cent. $CaCO_3$ *+ 15 per cent. of the same solution as in the second experiment.*

Cultures were incubated for 30 days at 25° C. Results are found in Table V.

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[1] Prepared from ground oat straw by boiling for 1 hour with 2 per cent. H_2SO_4, filtering, washing till free from acid, and drying.

Table V. *Decomposition of cellulose of acid-extracted oat straw in sand with neutral mineral salt solution, by bacteria and fungi.*

Culture	% of added cellulose decomposed	NO$_3$-N mg. per 100 gm. of sand	NO$_3$-N resorbed (mg.)
Vibrio B.M.	57·9	0·0	11·4
Vibrio H.M.	50·0	0·0	11·4
Vibrio W. 6	47·4	0·0	11·4
Vibrio G.St.	57·9	0·0	11·4
Vibrio from water	68·4	0·0	11·4
Coccospora sp.	63·2	0·0	11·4
Mycogone nigra	63·2	0·0	11·4
Botryosporium sp.	86·8	0·0	11·4
Stachybotrys sp.	81·8	0·0	11·4
Control	—	11·4	—

All the cellulose-decomposing vibrios as well as the fungi were capable of decomposing the lignified cellulose. *Stachybotrys* and *Botryosporium* were most active. The cellulose:nitrogen ratio appeared wider here than in the case of pure cellulose probably because the acid-treated straw still contained some proteid material, which may have served as nitrogenous food.

In some of the sand cultures of fungi on pure cellulose the medium became dark coloured at the end of the experiment. This suggested the formation of humic substances from the cellulose, a much discussed question which cannot be reviewed in detail here, but which may have a bearing on the formation of resistant nitrogenous humus during the decomposition of manure on the soil, as shown in a previous paper(12). A qualitative test for *a*-humus was made by extracting 20 gm. of dry sand (from experiments 2 and 3) with 20 c.c. 2·5 per cent. NaOH, in the autoclave, filtering, and acidifying the extract with HCl.

Table VI. *Production of humus-like substances by cellulose-decomposing organisms in sand culture.*

Organism	Colour of NaOH-extract	Precipitate with HCl
Vibrio prima	Pale yellow	None
Vibrio napi	,,	,,
Vibrio B.M.	,,	,,
Vibrio W. 6	,,	,,
Vibrio H.M.	,,	,,
Spirochaeta cytophaga	Golden	,,
Vibrio from water	Light yellowish brown	,,
Vibrio G.St.	Pale yellow	,,
Coccospora sp.	Yellowish brown	,,
Monosporium sp.	,,	,,
Trichoderma sp.	Light brown	Trace, brownish
Botryosporium sp.	Yellowish brown	Trace, yellowish
Mycogone nigra	Coffee brown	Brown, flocculent
Stachybotrys sp.	Purplish black	Black, flocculent

The data in Table VI show that the cellulose-decomposing bacteria did not form any such substances (as was also found by du Toit(6)), and the same is the case with some of the fungi, but two of these, *Mycogone* and *Stachybotrys*, formed a detectable amount of matter possessing the external characters of α-humus, or humic acid: an amorphous, dark brown to black material, soluble in alkalies and precipitated by acids. Enough sand-cellulose mixture was available to make a quantitative analysis. This gave the results shown in Table VII.

Table VII. *Formation of humus-like substances by fungi in sand culture.*

Organism	Sand extracted (gm.)	Humus obtained (gm.)	% of N in humus
Mycogone nigra	70	0·016	4·0
Stachybotrys sp.	90	0·036	5·9

The percentage of N in the humus-like material was thus of the same order as in soil humic acid. The fungi also form humus when growing in sterilised soil, as shown by another experiment. A light sand soil from Tunstall, of pH 4·9, received additions of 2 per cent. filter-paper, a source of N, and water to bring the soil up to 75 per cent. of its water-holding capacity. Portions of 150 gm. were placed in round 300 c.c. flasks, sterilised, and inoculated with four cellulose-decomposing fungi. The following series was run:

Addition besides filter-paper	Inoculation
Nothing	Sterile (control)
0·25 % (NH$_4$)$_2$SO$_4$	*Trichoderma* sp.
	Mycogone nigra
0·25 % NaNO$_3$ + 1·0 % CaCO$_3$	*Stachybotrys* sp.
	Botryosporium sp.

After 50 days' incubation at 25° C. the results shown in Table VIII were obtained.

It is seen that *Trichoderma* and *Botryosporium*, which did not produce significant quantities of humus in sand, gave only a very slight increase in the amount of α-humus, but a considerable increase in its N percentage. This is probably due to the extraction of some protein from the mycelium. *Mycogone nigra* gave a distinct increase in the amount of α-humus, and its N percentage shows less increase (the increase corresponds to a content of 5 per cent. N in the extra humus which the fungus has formed). Finally, *Stachybotrys* gave a very notable increase in the amount of α-humus and a corresponding increase in its N percentage. This experiment with pure cultures confirms the statement of Waksman(27) that

a nitrogenous α-humus can be formed from pure cellulose decomposing in sand, and offers us an explanation of the extra formation of α-humus during manure decomposition in soil, since the two fungi studied here are active in the decomposition of straw in neutral and alkaline soils. The humus formation is not, however, necessarily dependent on the presence of cellulose, since the humus-like material is a constituent of the protoplasm of these and other micro-organisms and can be formed from other carbonaceous food. This leads us to the problem of humus formation through the decomposition of microbial protoplasm, which will be dealt with in another contribution.

Table VIII. *Production of α-humus by pure cultures of cellulose-decomposing fungi in sterile soil.*

Organisms	α-humus (%)	N in α-humus (%)*	Appearance of cultures
Control, sterile	0·21	3·39	—
Trichoderma sp.	0·25	4·47	After 1–3 weeks' abundant mycelial development and sporulation, after 4–6 weeks' growth hardly visible
Mycogone nigra	0·28	3·84	After 1–3 weeks' strong mycelial development, which later disappears
Stachybotrys sp.	0·40	4·20	Very little mycelial development, but soil coloured dark by spores
Botryosporium sp.	0·23	4·41	Visible mycelial growth, but less strong than that of *Trichoderma* and *Mycogone*

* Average of two parallel determinations. Method of humus determination described in the previous paper.

Summary.

1. Addition of farmyard manure to soil gives rise, in laboratory experiments, to an abundant development of cellulose-decomposing bacteria of the genus *Vibrio* in approximately neutral soils (pH 6·5–7·0). In faintly acid soils (pH 5·7–6·2) these organisms develop less abundantly, and are partly replaced by *Spirochaeta cytophaga*. At lower pH values only the fungi are active in the decomposition of cellulose. Similar results were obtained by adding filter-paper or straw to soils of different reactions. Of the fungi, *Trichoderma* and *Penicillium* appear more active in acid soil, whereas other forms, among others *Mycogone nigra*, *Stachybotrys* sp., *Coccospora agricola* (?), and *Botryosporium* sp. seemed prominent in neutral soil.

2. The vibrios, of which four strains were studied in pure culture, are very sensitive to acidity. They fail to develop in the pH interval

$6 \cdot 0$–$6 \cdot 4$, and have an optimum at pH $7 \cdot 1$–$7 \cdot 6$. *Spirochaeta cytophaga* appears to be slightly more resistant to acidity, being able to grow at pH $5 \cdot 6$–$6 \cdot 0$.

3. The bacteria as well as the fungi are capable of decomposing the lignified cellulose of straw.

4. The nitrogen requirements of the cellulose-decomposing bacteria are not smaller than those of the fungi. The ratio of decomposed cellulose to assimilated nitrogen in pure cultures ranges between 25:1 and 54:1 without any clear difference between the two groups of organisms.

5. Cellulose-decomposing bacteria do not form humus-like compounds when growing on filter-paper in sand culture, but at least two of the fungi, *Mycogone nigra* and *Stachybotrys* sp., form such compounds in sand as well as in sterile soil.

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CALCIUM AND HYDROGEN ION CONCENTRATION AND THE INTERFACIAL TENSION OF PYRETHRUM EXTRACTS.

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(With Four Text-figures.)

CONTENTS.

INTRODUCTION.

THE modern theory of emulsions developed by Bancroft[1] postulates an adsorbed film round the globules; in the case of a soap being the emulsifier, the hydrocarbon chain is orientated towards the oil phase, the carboxyl group towards the aqueous phase. That alkalis have a marked effect in promoting emulsification has long been known; Donnan[4] showed that the formation of a soap was responsible, as mineral oil freed from fatty acid had the same interfacial tension in alkaline as in neutral solutions. While no one physical property can be held to account for the stability of emulsions, the interfacial tension gives generally a good indication of the probability of emulsification and, for its measurement, a drop-weight technique has been extensively used.

Hartridge and Peters[7] confirmed this method by comparison with the capillary height and a modified ripple method. Using olive oil dropped into buffer solutions, they examined the effect of the pH of

the aqueous phase on the interfacial tension; the tension was found to fall with increasing alkalinity, becoming immeasurable at pH 9·0.

With extracts of the insecticidal plant, *Pyrethrum cinerariaefolium*, the problem of emulsification is complicated by the fact that the two poisons present, the pyrethrins, are sensitive to alkali, having been shown by Staudinger and Ruzicka[11] to be esters. An exact adjustment of the hydrogen ion concentration may, therefore, be necessary to secure the optimum conditions for the stability of both the poisons and the emulsion. Accordingly, an examination was made of the effect of the hydrogen ion concentration on the interfacial tension of pyrethrum extracts against aqueous solutions.

In addition, the influence of calcium ions was studied, as hard waters frequently have to be used in making up insecticidal washes and the soaps of divalent cations tend to promote the water-in-oil type of emulsion and even in very small amounts may cause inversion.

METHODS AND TECHNIQUE.

Drop-weight technique. Measurements were made by a drop-weight method and in all the experiments the oil was run into the aqueous phase. The apparatus consisted of a 2 c.c. graduated pipette turned up at the bottom and joined to a short piece of thick-walled capillary tubing, the free end of which was ground as smooth as possible. The upper end of the pipette was fixed by means of pressure tubing to a grooved tap, the pipette filled by suction and the oil allowed to run out by its own gravity, the rate being controlled by partially closing the tap. The same pipette was used throughout and was shown to have a perfectly uniform bore. No standardisation for any error in the absolute volumes of the readings was applied, as the constant for the apparatus automatically includes this. The temperature was controlled by immersing in a beaker of water kept at 18° C. and in all the observations the drop was allowed to break away very slowly, the time per drop being between 45 and 90 seconds.

Before allowing the oil to run out, the tip was freed from oil and wetted by rubbing gently with a plug of cotton-wool, previously moistened with the aqueous solution, and forcing the oil a short way down the capillary by slight pressure. In this way it was ensured that the drop came from the inner edge of the tip. For very large drops about 1 c.c. of oil was run out. For smaller drops 70 to 150 of them were run out, an approximate value obtained by counting 5 to 10 drops and noting the volume, at intervals the number calculated to the nearest integer by taking readings as the drop broke away, and the final value obtained

by dividing the total volume by the total number of drops. When the drops were so small that there was some doubt as to the nearest whole number, 30 to 40 of them were counted directly.

Methods of standardisation and calculation. The apparatus was standardised by measuring the drop volume for benzene against water. The value for the interfacial tension of benzene-water was taken as 34·61 dynes/cm. interpolated for 18° C. from the accurate measurements of Harkins and Humphery[6]. The constant was calculated from the following equation given by Hardy[5], from which the values for the interfacial tension, T_{AB}, were obtained:

$$T_{AB} = \frac{b(D_B - D_A)g}{rf}.$$

b = volume of 1 drop in c.c.

D_B = density of aqueous layer.

D_A = density of oil layer.

g = gravity (981).

r = radius of tube.

f = empirical factor.

Although this method of standardisation is open to criticism, it has been used in similar investigations and is probably valid where only comparative accuracy is required.

Material. The solutions were made up by diluting a highly concentrated extract of pyrethrum, prepared by treatment of the flower heads with low-boiling petroleum ether and subsequent concentration *in vacuo* at a low temperature. This extract was analysed by the rapid method of Tattersfield and Hobson[13] and found to contain 4·5 per cent. of pyrethrin I. This amount corresponds to approximately 9 per cent. of mixed pyrethrins and 45 per cent. of oleo-resinous matter, since the pyrethrins are usually present in about equal amount and constitute approximately 20 per cent. of the material extracted by petroleum ether (Tattersfield, Hobson and Gimingham[12]). A high-grade sample of semi-refined white spirit was used as the solvent.

Relationship between the interfacial tension and hydrogen ion concentration.

Determinations of the interfacial tension were made against fully buffered solutions over a range of hydrogen ion concentration. The effect of the addition to the oil phase of an emulsifying agent, agral W.B., was

also investigated. The following solutions were made up in semi-refined white spirit and tested:

(a) 5 per cent. pyrethrum extract.
(b) 5 per cent. pyrethrum extract + 10 per cent. agral W.B.
(c) 10 per cent. agral W.B.
(d) Solvent—semi-refined white spirit.

The buffers were prepared from the "universal buffer" mixture recommended by Prideaux and Ward[9], which consists of a solution of phenyl-acetic, phosphoric and boric acids, and yields, by the addition of varying amounts of soda, buffers over a range from pH 3·1 to 11·0. This buffer mixture possessed the advantage that it could be used throughout the experiments, but had the drawback that it was not without influence on the interfacial tension; that this effect was not serious will be shown later.

Table I. *The interfacial tension of solutions of pyrethrum extracts against buffers of varying pH.*

Oil phase	pH of buffer	Drop volume (c.c.)	Tension (dynes/cm.)
5 per cent. of pyrethrum extract in semi-refined white spirit	3·1	0·0122	8·32
	4·7	0·0104	7·12
	6·0	0·00910	6·25
	7·0	0·00815	5·62
	8·0	0·00393	2·72
	9·0	Immeasurable	—
5 per cent. of pyrethrum extract and 10 per cent. of agral W.B. in semi-refined white spirit	3·1	0·00591	3·70
	4·7	0·00544	3·42
	6·0	0·00523	3·30
	7·0	0·00250	1·58
	8·0	0·00067	0·43
	9·0	Immeasurable	—
10 per cent. of agral W.B. in semi-refined white spirit	3·1	0·00630	3·97
	4·7	0·00588	3·73
	6·0	0·00549	3·50
	7·0	0·00289	1·85
	8·0	0·00070	0·45
	9·0	Immeasurable	—
Solvent: semi-refined white spirit	3·1	0·0497	34·3
	4·7	0·0577	40·0
	6·0	0·0634	44·1
	7·0	0·0693	48·4
	9·0	0·0530	37·2
Solutions of pyrethrum extract in semi-refined white spirit at the following concentrations: 5 % 10 % 20 % 50 % 75 % 100 %	7·0	0·00815 0·00604 0·00569 0·00661 0·00755 0·00952	5·62 4·12 3·78 4·06 4·31 5·03

Results. The results are contained in Table I and represented diagrammatically in Fig. 1. The figures in Table I show that the addition of pyrethrum extract alone lowers the interfacial tension very considerably, even against acid solutions, and there must be present in the extract substances possessed of high surface activity. The extract consists of the pyrethrins, fatty and resinous material which may be conveniently described as oleo-resins, and petroleum ether. The last-named can have little effect, as its interfacial tension against water is not materially lower than that of semi-refined white spirit. Whether the
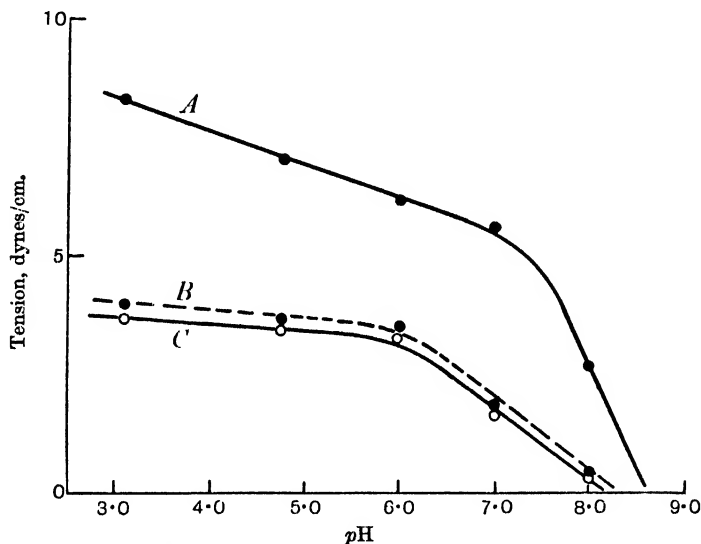
Fig. 1. *p*H-interfacial tension curves for oil against buffers. Solutions in semi-refined white spirit: A, 5 per cent. of pyrethrum extract; B (broken line), 5 per cent. of pyrethrum extract + 10 per cent. of agral W.B.; C, 10 per cent. of agral W.B.

active substances are the pyrethrins or the accompanying oleo-resins could not be determined as, unfortunately, samples of the pure pyrethrins were not available.

The effect of *p*H on the interfacial tension of the solution of pyrethrum extract may be seen from the curves in Fig. 1; with an increasing alkalinity the tension falls, steadily at first from *p*H 3·1 to 7·0, then more rapidly until at *p*H 9·0 stream formation occurs and the oil passes spontaneously into an emulsion. The addition of 10 per cent. agral W.B. further lowers the interfacial tension in the extreme acid region. From *p*H 3·1 to 6·0 the tension of this solution drops very slightly, and from *p*H 6·0 to 8·0 more rapidly. The presence of the agral W.B. appears to

produce a lower tension and less sensitivity to the reaction of the aqueous phase than in the case of the pyrethrum extract alone; with both the tension vanishes at approximately the same pH. The solution of 10 per cent. agral W.B. without pyrethrum extract possesses at all reactions a slightly higher tension than that with pyrethrum extract included, but the two curves follow each other very closely.
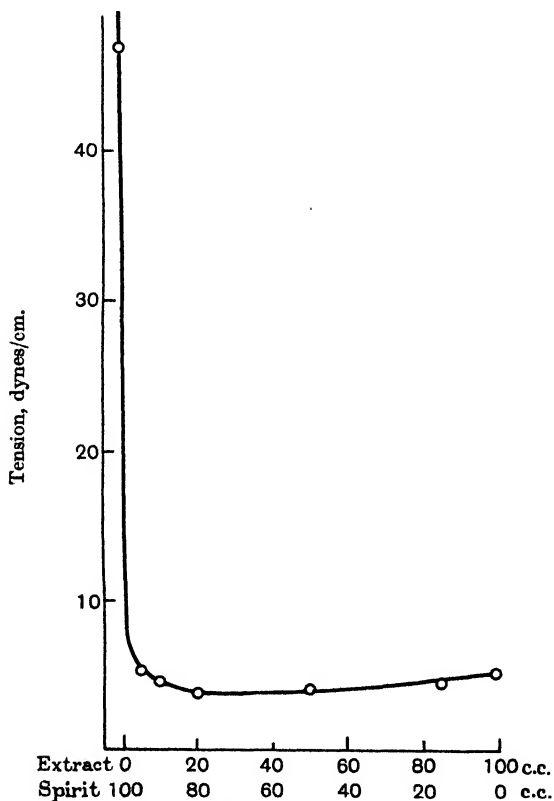
Fig. 2. Interfacial tension of mixtures of pyrethrum extract and semi-refined white spirit against buffer pH 7·0.

The results for semi-refined white spirit alone, given in Table I, show that the buffer mixture has probably influenced the interfacial tension, which is seen to fall with departure from neutrality on either side. Donnan(4) has shown that the tension of pure mineral oils is not affected by changing the hydrogen ion concentration. In this case the effect is possibly due to traces of fatty acid in the semi-refined white spirit, but

more probably to the presence in the buffer of phenyl-acetic acid. It is, however, not likely that the tensions of the solutions of pyrethrum and of agral W.B. are at all disturbed, except possibly in the extreme acid region, since a weakly active substance is unlikely to exert much influence in the presence of more active ones.

THE EFFECT OF INCREASING THE CONCENTRATION OF PYRETHRUM EXTRACT IN THE SOLUTION.

The lowering of the interfacial tension of semi-refined white spirit by the addition of small amounts of pyrethrum extract suggested that it might be advantageous to use more concentrated solutions. The effect of the concentration of the pyrethrum extract was, therefore, studied, using a buffer of pH 7·0. The results, given in Table I and Fig. 2, show that the tension falls with increasing concentration to a minimum between 10 and 50 per cent. but the decrease is not large. It is possible that the rise in the tension with concentrations over 20 per cent. is only apparent; with increasing concentration the viscosity increases and it is well known that the drop-weight method breaks down with viscous liquids owing to the distortion of the drops, though in this case the drops had a normal appearance.

The lowering of the interfacial tension produced by increasing the concentration up to a certain limit seems hardly sufficient to compensate for the following disadvantage. As the petroleum is not without toxic action and enhances the effect of the more expensive pyrethrum, this increase of toxicity would be partly lost by using a more concentrated solution of pyrethrum extract since, for the same amount of poison in the emulsion, the less petroleum solvent would be present the more concentrated the solution.

THE EFFECT OF THE PRESENCE OF CALCIUM SALTS.

Whereas the soaps of monovalent cations decrease the interfacial tension between the oil and water and promote the formation of stable oil-in-water emulsions, the soaps of the divalent cations favour the water-in-oil type. Determinations were, therefore, made of the interfacial tension of solutions of pyrethrum extract against a hard water containing calcium, to which various substances were added.

For these experiments Harpenden tap-water was used; this is a typical hard water, in which emulsions are only formed with difficulty. The hardness of the water was found to be as follows: 27·6° temporary

hardness and 30·1° total hardness as calcium; only traces of magnesium were present. Solutions were made up to contain various normalities of sodium hydroxide, carbonate and phosphate. Determinations were made of the pH colorimetrically using standards prepared from Prideaux and Ward's buffer mixture. The interfacial tension was measured with solutions in semi-refined white spirit containing 5 per cent. of the pyrethrum extract, both with and without the addition of 10 per cent. of agral W.B.

The aqueous solutions were not fully buffered, but it appears unlikely that the pH at the interface would be seriously disturbed as neither the pyrethrum extract nor the agral W.B. are strongly acid, and the concentration of each was low. Hartridge and Peters(7), working with pure oleic acid dropped into $N/100$ soda, found that the drops were small if formed rapidly and large if formed slowly; this abnormality they explained as the result of neutralisation when the drop formed slowly. Changing the rate of drop formation did not have this effect on the drop volume in my experiments, and there can be little doubt that no significant changes in the pH occurred at the interface.

The results with Harpenden tap-water are given in Table II. In Fig. 3 the values for the interfacial tension are plotted against the hydrogen ion concentration, the curves obtained with the buffers (free from calcium) being repeated for purposes of comparison.

Table II. *The interfacial tension of solutions of pyrethrum extract in semi-refined white spirit against Harpenden tap-water containing added solutes.*

Aqueous phase		Tension: dynes per cm.	
Solute	pH	5 % pyrethrum extract	5 % pyrethrum extract + 10 % agral W.B.
— (tap-water)	6·9	7·18	4·76
0·04 N NaCl	6·9	—	5·00
0·01 N Na$_2$CO$_3$	8·4	5·59	2·79
0·02 N ,,	9·5	Immeasurable	Immeasurable
0·04 N Na$_2$HPO$_4$	7·6	5·20	2·76
0·12 N ,,	8·3	3·02	0·6
0·001 N NaOH	8·5	7·3	4·8
0·005 N ,,	9·8	6·7	4·5
0·007 N ,,	10·1	5·4	1·6
0·010 N ,,	10·9	Immeasurable	Immeasurable
0·5 % agral I	—	0·3	—

The effect of a small amount of calcium salt is well illustrated by the result obtained with the solution of pyrethrum extract and agral W.B. against untreated tap-water in which the concentration of calcium was 0·003 moles per litre (corresponding to pCa 2·5) and the pH 6·9. The interfacial tension was found to be 4·76 dynes, the value for the buffer

solution of this pH being 1·8 dynes (interpolated). With this solution the interfacial tension appears to be more sensitive to the presence of calcium ions than of hydrogen ions, since the tension against the most acid buffer tested (pH 3·1) was 3·7 dynes and from the flatness of the curve in this region would probably be under 4·0 dynes at pH 2·5; with tap-water of pCa 2·5 the value was 4·76 dynes. When the agral W.B. is absent, hydrogen ion is the more effective as the tension with the buffer at pH 3·1 (8·32 dynes) is higher than with tap-water of pCa 2·9 (7·18 dynes).
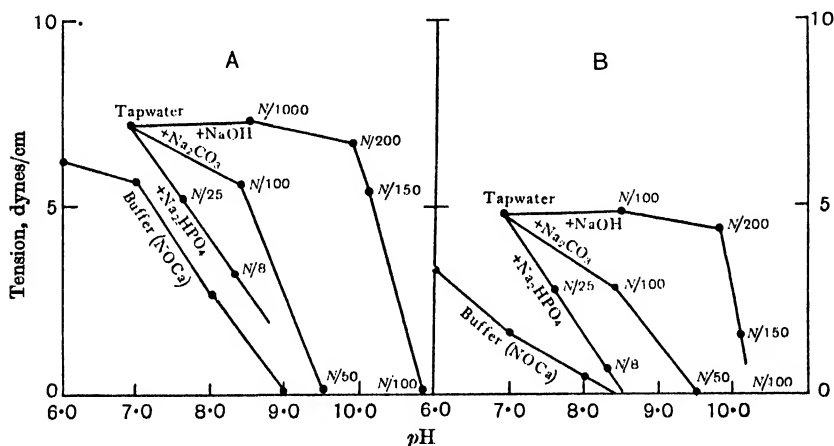
Fig. 3. pH-interfacial tension curves for oil against tap-water containing calcium and added salts and against calcium-free buffers. A, 5 per cent. solution of pyrethrum extract in semi-refined white spirit; B, 5 per cent. solution of pyrethrum extract in semi-refined white spirit + 10 per cent. agral W.B.

The results obtained with tap-water containing a wetter, agral I, raises an interesting point. It will be seen from Table II that the addition of agral I has decreased the tension of the pyrethrum extract solution against tap-water from 7·18 to 0·3 dynes. This effect is probably due to the lowering of the surface tension of the water and adsorption of the agral I at the interface. When mixtures of pyrethrum extract solutions and water containing agral I were gently shaken, they passed readily into an apparent emulsion, which broke just as readily in one or two minutes. Evidently agral I lowers the tension without stabilising the emulsion.

An examination of Fig. 3 shows that increasing the alkalinity by adding sodium hydroxide to the tap-water lowers the interfacial tension; the results with sodium carbonate and phosphate solutions indicate that

the tension can be reduced at lower hydroxyl ion concentrations by precipitating the calcium. In order to study more closely the relationship between the interfacial tension and the concentration of calcium and hydroxyl ions in the aqueous solution, the amounts of calcium present in the solutions used were calculated.

The methods of calculation and the data employed were those given by Hastings, Murray and Sendroy [8] and Sendroy and Hastings [10] in their studies on the solubilities of calcium carbonate and calcium phosphates in biological fluids. The values for the concentrations of carbonate ions were calculated from the amounts of carbonate known to be present without reference to the atmospheric CO_2 tension, since the solutions were not brought into equilibrium with the air and could absorb but little carbon dioxide during the experiments. In the solutions containing sodium hydroxide, which gave little or no precipitate unless heated and were evidently supersaturated with calcium carbonate, the original amount of calcium was assumed to be present. No precautions were taken to prevent supersaturation in any of the solutions; however, since the sodium carbonate and phosphate solutions were prepared by diluting strong solutions in tap-water, the solid phase was present at the moment of preparation. It has also been tacitly assumed that the presence of the precipitate was without influence.

Table III. *The interfacial tension of solutions of pyrethrum extract in semi-refined white spirit and the ratios $\dfrac{[Na^+]}{[Ca^{++}]}$ and $\dfrac{[OH']}{[Ca^{++}]}$ in the aqueous phase.*

| Tap-water solution | | | | | | Tension: dynes per cm. | |
| | Nor-mality | a pCa^{++} | b pO$'$H | $\log \dfrac{[Na^+]}{[Ca^{++}]}$ | $a-b$ $\log \dfrac{[O'H]}{[Ca^{++}]}$ | 5 % pyrethrum extract | 5 % pyrethrum extract + 10 % agral W.B. |
Solute							
Na$_2$CO$_3$	0·01	4·4	5·7	2·4	−1·3	5·6	2·8
,,	0·02	5·4	4·6	3·7	+0·8	Immeasurable	Immeasurable
Na$_2$HPO$_4$	0·04	5·1	6·5	3·5	−1·4	5·2	2·8
,,	0·12	5·6	5·8	4·8	−0·2	3·0	0·6
NaOH	0·001	?2·5	5·6	−0·5	−3·1	7·3	4·8
,,	0·005	?2·5	4·3	0·2	−1·8	6·7	4·5
,,	0·007	?2·5	4·0	0·3	−1·5	5·4	1·6
,,	0·01	?2·5	3·2	0·5	−0·7	Immeasurable	Immeasurable
—	—	2·5	7·2	?	−4·7	7·2	4·8

Table III gives the concentrations of sodium, calcium and hydroxyl ions, the logarithms of the ratios of sodium and of hydroxyl to calcium ion concentrations, and the interfacial tensions. Evidently, neither the concentration of calcium or hydroxyl ion nor the ratio of sodium to

calcium ions bears any relation to the interfacial tension figures; on the other hand, the values for the logarithm of the ratio of hydroxyl to calcium ion concentration appear to show a definite correlation. This is illustrated in Fig. 4, in which the interfacial tension is plotted against
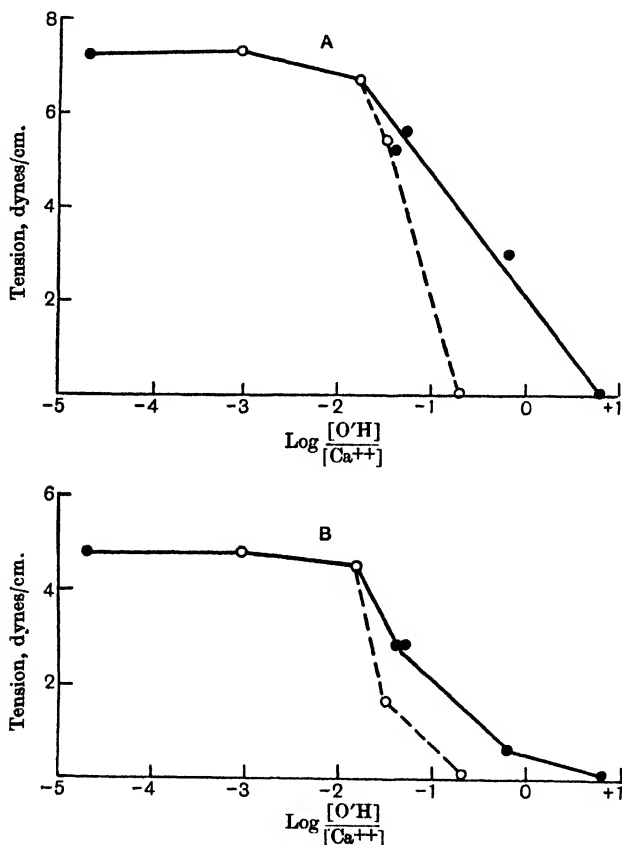
Fig. 4. Interfacial tension and the logarithm of the ratio of calcium to hydroxyl ions in the aqueous phase. Oil phase: A, 5 per cent. solution of pyrethrum extract in semi-refined white spirit; B, 5 per cent. solution of pyrethrum extract in semi-refined white spirit + 10 per cent. agral W.B. Aqueous phase: tap-water with added NaOH, Na_2CO_3, Na_2HPO_4. Results with NaOH ringed and, where they diverge, shown by broken line.

$\log \dfrac{[O'H]}{[Ca^{++}]}$; with the exception of the values for the sodium hydroxide solutions which are treated separately, the tension falls steadily as the logarithm of the ratio increases from -1 to $+1$ at which point the

tension vanishes. The curves obtained are not dissimilar to those (Fig. 1) in which the interfacial tension is plotted against the pH in the absence of calcium.

In the case of the sodium hydroxide solutions, as the figures for pCa are uncertain and supersaturation has been shown to be present, it is not surprising that the results are analogous. Nevertheless, it is possible that at the higher concentrations the critical ratio may not be the same. The effect of calcium ions is presumably due to their adsorption, which would be relatively less at high concentrations, and the ratio of hydroxyl ion to calcium ion adsorbed at the interface, rather than the ratio in the solution, is probably the determining factor. An alternative possibility is that, at the higher alkalinities, changes such as hydrolysis may occur in the oil at the interface.

DISCUSSION.

It has been shown that the addition of a pyrethrum extract lowers the interfacial tension of semi-refined white spirit against aqueous solutions to a marked degree (Table I). Whether this result is due to the pure poisons, the pyrethrins, or to the concomitant oleo-resinous matter, has not been determined, but it seems likely that the resins are mainly responsible as they are known to possess good emulsifying properties.

With increasing alkalinity in the aqueous phase the tension decreases (Fig. 1). Hartridge and Peters[7], working with olive oil, found that a rapid fall in the tension occurred at pH 5·0 and related this to the change in volume of the carboxyl group, which occurs at this pH. With pyrethrum extracts the tension falls gradually from pH 3·0 to pH 7·0 and then more rapidly; that the whole change is spread over a wider range of pH and is less abrupt than with olive oil, may be explained by supposing that an acid group of a different nature is operative. The results obtained when agral W.B. is added (Fig. 1) confirm this view; thus, the introduction of a different acid group completely alters the relationship between the interfacial tension and the reaction of the aqueous phase. Further, the more active agral W.B. seems to supersede the effective constituent in the pyrethrum extract, as the curves for solutions of the two together and of the agral W.B. alone are almost identical.

The presence of calcium salts in the aqueous phase raises the tension of pyrethrum solutions (Fig. 3), and the addition of agral W.B. to the oil decreases the effect of calcium ions as it does that of hydrogen ions. line salts counteract the influence of calcium salts and the order of

efficiency at the same pH has been found to be sodium phosphate, sodium carbonate and sodium hydroxide. This is also the decreasing order both of the valency of their anions and of their power to precipitate calcium salts.

An examination of the concentrations of different ions in these solutions has shown that the ratio of hydroxyl to calcium ions can be correlated with the interfacial tension values. It is of interest to note that both Clowes[3] and Bhatnagar[2] came to similar conclusions, using different oil emulsions and relatively high concentrations of divalent salts. Clowes showed that sodium salts counteracted the effect of calcium salts on olive-oil emulsions, the critical ratio being 100 mols of sodium chloride, or 4 mols of sodium hydroxide, to 1 mol of calcium chloride. Bhatnagar found a critical ratio of 4 mols of potassium hydroxide to 1 mol of barium nitrate with paraffin-oil emulsions. For the emulsions of pyrethrum extract (Table III) the critical ratio of hydroxyl to calcium ion appears to lie between 1 and 10 when the calcium concentration is low. Clowes attributed the effect of sodium salts to the adsorption of the anion, the cation playing no part. My experiments confirm this view since the ratio of sodium to calcium ion concentration appears to have no effect on the tension (Table III), even when it rises to over 1000.

It is not certain that softening agents improve oil emulsions in hard waters only by lowering the interfacial tension. This value measures the resistance to mixing of two immiscible liquids and, when it approximates to zero, the two liquids will readily pass on shaking into an emulsion which may or may not be stable. The interfacial tension, therefore, cannot be regarded as a reliable index of the stability of the resulting emulsion. In the case of the insecticidal emulsions it is essential to have information as to the breaking properties of the emulsion; an account of direct investigations on this question will be included in a separate publication.

The author is indebted to Dr F. Tattersfield for suggesting this investigation and its close bearing on the emulsification of pyrethrum extracts and for his active interest in the work and the writing of this paper.

Summary.

1. The addition of a pyrethrum extract to a petroleum solvent, semi-refined white spirit, considerably lowers its interfacial tension against water. The tension also depends upon the reaction of the aqueous phase, decreasing as the alkalinity increases.

2. The addition of agral W.B. to a solution of pyrethrum extract further lowers the interfacial tension more especially against acid solutions, thereby decreasing the sensitivity of the tension value to the pH of the aqueous phase.

3. The presence of calcium salts in the aqueous phase raises the interfacial tension of solutions of pyrethrum extract.

4. Alkaline salts counteract the effect of calcium salts, and the resulting tension values can be correlated with the ratio of calcium to hydroxyl ion concentration.

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THE EVALUATION OF PYRETHRUM FLOWERS (*CHRYSANTHEMUM CINERARIAEFOLIUM*).

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(With Plate I and Three Text-figures.)

CONTENTS.

INTRODUCTORY.

THE extended use of pyrethrum (*Chrysanthemum cinerariaefolium*) as an insecticide in recent years and the necessity of increasing the production of this plant have demanded better methods of evaluation of the active principles. This demand will be accentuated if, as seems probable, an intensive campaign of selection for higher content of the pyrethrins is undertaken. The work of Staudinger and Ruzicka[4] on the constitution of the pyrethrins has already led to suggestions of methods of chemical analysis associated with the names of Staudinger and Harder[5], Tattersfield, Hobson and Gimingham[6] and of Gnadinger and Corl[1]. Although further work is still needed to ascertain whether one or all of these methods are suitable for detecting the loss of toxicity associated with exposure of the finely powdered flowers and certain extracts to the

atmosphere, the methods proposed may be regarded as satisfactory for the recently harvested crop or carefully stored flowers. None are foolproof, and there would appear to be conditions under which relatively inaccurate results may be obtained. We propose in this paper to examine some of the difficulties associated with certain of these methods. In addition, it is desirable to have methods applicable to the produce of a single plant and, if possible, for the evaluation of a single flower head— we therefore also suggest two new methods which, although perhaps not susceptible to the same degree of accuracy as the older methods, require much less material and are likely to be of use for this purpose.

The active principles, namely pyrethrins I and II, are associated in the plant with other organic compounds, and are esters resulting from the condensation of a ketonic alcohol (pyrethrolone) and two acids, one monobasic, volatile in steam, and the other dibasic and soluble in water.·

Pyrethrin I   Pyrethrin II
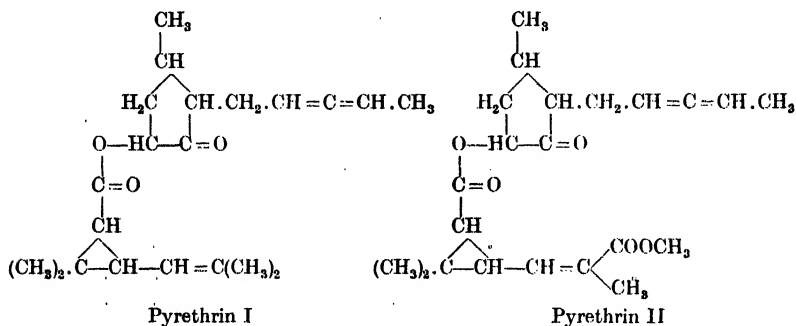
It is not outside the range of possibility that in the flowers small amounts of the pyrethrolone may exist associated with other acids than the two pyrethrin acids, and that the latter may in small amount be condensed with some other alcohol. It is, therefore, of some importance to ascertain the degree of concordance between the results obtained in the determination of the active principles by means of the estimation of the acidic portion and those by the determination of the ketonic-alcohol fraction of the pyrethrin molecules. Tattersfield, Hobson and Gimingham[6] reduced the two methods originally suggested by Staudinger and Harder[5] to a microscale and compared them; relatively close agreements were obtained between the acid method and the semicarbazone method, but it was felt that the latter was too complicated and tedious for general use. More recently Gnadinger and Corl have published a much more rapid method of ascertaining the active principles depending upon their reducing properties, which are associated with the

pyrethrolone portion of the pyrethrin molecules. We have compared the results obtained by these methods, and although for samples of high pyrethrin content certain modifications will have to be suggested, so far results have been obtained which are in comparatively close agreement. Concordance of a very high order is hardly to be expected, however desirable, but it must also be recognised that small differences in pyrethrin content can only be detected, if at all, with great difficulty by means of the biological insecticide tests at present available. Moreover, owing to wide variations in insect resistance to the toxic effects of pyrethrum, even with the same species, an ample margin of safety should always be allowed in spraying practice, and this tends partially to off-set the higher value of a sample with only a slightly greater poison content than another. The agreements obtained are, in our opinion, sufficiently good therefore to warrant their use for evaluating samples, for the standardisation of extracts, and to standardise the newer methods here suggested.

In carrying out a quantitative investigation on the change in amount of the active principles during the development of the flowers from the small bud stage to the fully open flowers, there was found to be a considerable variation from plant to plant grown from selected seed from the same source, and with the flowers at the same stage of development. The analysis of a fairly large number of plants had to be undertaken in order, as far as possible, to allow for these variations, and the occurrence of one or two plants of an exceptional nature might easily have led to erroneous deductions as to the stage at which the average pyrethrin content was at a maximum. It was thus decided to search for a method requiring so small an amount of material that the whole, or at any rate a large part, of such an investigation could be carried out on the flowers derived from one plant.

It was found that the flowers of certain plants were very rich indeed in pyrethrin content and that for such samples the acid method as outlined by Tattersfield, Hobson and Gimingham (6) tended to give low results. Some modification was obviously needed. The modification suggested is, in the main, that the weight taken for extraction should be reduced for samples containing more than a certain percentage of the total pyrethrins, and that greater care be taken to secure their complete hydrolysis. An alternative method which could be used for rapidly ascertaining, if only approximately, this percentage would be of value, which would be all the greater if only a small amount of the sample were required. If necessary the acid method could then be carried out to determine the actual amount of the two separate constituents.

The acid method.

In the course of an investigation by one of us of the flower heads from separate plants, a degree of variation in percentage pyrethrin content was observed. In a number of cases this value was higher than we had hitherto observed or had thought was to be expected when the original acid method was devised. It was decided to check these values and, in order to assure complete hydrolysis of the esters, to use a smaller weight of flower heads and a greater relative amount of alkali for the hydrolysis. The results, particularly for pyrethrin II, frequently came out higher than when the method as originally described was employed.

In Table I are recorded data indicating the kind of discrepancy that may occur.

Table I.

Sample no.	Pyrethrin I (%)	Pyrethrin II (%)	Total pyrethrins (%)	Weight used (gm.)
G. 1	0·69	0·65	1·34	10·0
	0·68	0·73	1·41	5·0
	0·68	1·02	1·70	2·5
H. 10	0·50	0·71	1·21	10·0
	0·52	0·92	1·44	5·0
	0·50	1·00	1·50	2·5
G. 4	0·96	0·72	1·68	5·0
	0·95	0·94	1·89	2·5
G. 9	0·61	0·82	1·43	10·0
	0·63	1·06	1·69	5·0
	0·63	1·14	1·77	2·5

For these high samples where 10 gm. has been employed, the values are on the low side, and although this may be due to imperfect hydrolysis or incomplete extraction of the dicarboxylic acid or to both, adsorption during filtration of the dicarboxylic acid after the distillation of the volatile acid appears also to be involved. In a previous investigation [6] it was noted that a small quantity of animal charcoal will adsorb practically the whole of the dicarboxylic acid and when, as in such samples as these, the acid is fairly highly concentrated in the residue after distillation, it may be partially held back on the cotton-wool pad. On the other hand, where 2·5 gm. is used, very careful titration indeed is required, as the smaller weight used leads to a proportionally greater experimental error. We have adopted in more recent work the following modifications of the original method. For samples of poor quality—below 0·7 per cent. total pyrethrins we have extracted with petroleum ether 10 gm., for

samples between 0·7 and 1·5 per cent., 5 gm. and for samples above 1·5 per cent., 2·5 gm. After taking down to a small bulk in a current of CO_2 and evaporating the residue in a vacuum desiccator, the residue is extracted with four lots of 2·5 c.c. each of gently warmed purified methyl alcohol, each of which is cooled and filtered into a 100 c.c. Kjeldahl flask through a pad of cotton-wool. A final washing with 2·5 c.c. cold methyl alcohol is made, a few drops of phenolphthalein in methyl alcohol are added and then, drop by drop till just alkaline, a solution of caustic potash in methyl alcohol of $N/1$ strength. A further 5 c.c. are added and the mixture refluxed for a full 8 hours. The methyl alcohol is taken off in partial vacuum with gentle warming (the temperature not being allowed to rise above 25° C.), the residue dissolved in water and the volatile acid distilled off in steam. The volume in the distillation flask should not be allowed to exceed 30 c.c. Two lots of 50 c.c. are distilled off and the acids in the first distillate extracted with two lots of 50 c.c. of petroleum ether, each extract being washed with 20 c.c. of distilled water. The two extracts are combined, evaporated on a water bath after addition of 20 c.c. of distilled water, and the residue titrated while warm with $N/50$ soda, the sides of the flasks being washed down towards the end with a little neutral methyl alcohol. From the titration the amount of pyrethrin I can be determined [6]. The second distillate of 50 c.c. may be extracted with petroleum ether; it should not show more than a trace of titratable acid. The hot aqueous residue in the distillation flask is treated with 0·2 gm. of calcium sulphate and, after standing overnight, filtered through a cotton-wool plug, washed three or four times with water and extracted exhaustively with sodium-treated ether in the apparatus already described [6]. In a rapid extractor 20 hours' extraction appears to be the minimum time necessary for complete extraction of the dicarboxylic acid in the case of samples of high pyrethrin content. After adding 20 c.c. of distilled water the ether is evaporated, the aqueous layer heated to boiling, cooled and filtered through a cotton-wool plug and the filtrate, after heating to boiling, titrated with $N/50$ soda.

1 c.c. $N/50$ alkali = 3·36 mg. monocarboxylic acid = 6·6 mg. Pyrethrin I
              = 1·98 mg. dicarboxylic acid   = 3·74 mg. Pyrethrin II.

The apparatus employed is precisely the same as that described by Tattersfield, Hobson and Gimingham [6]. The method requires considerable care if accurate results are to be obtained and, although the determination of pyrethrin I has not presented any great difficulty, the estimation of pyrethrin II is not free from technical difficulties and makes the process rather prolonged. We describe below a short method of

determining the total pyrethrins, which for flowers in the later stages of development, *i.e.* later than the small bud stage, has given results for the total pyrethrins in general concordance with both the acid and the Gnadinger and Corl methods. It is probable that by its use, combined with the present method of determining pyrethrin I, a sufficiently accurate value for pyrethrin II would be obtained in a shorter time and with less technical difficulty. This simplification is now under investigation.

The acid method and Gnadinger and Corl method compared.

We have tested a number of samples by both these methods, the results being given in Table II.

Table II. *Comparison of results by acid method and method of Gnadinger and Corl.*

	Acid method		Total pyrethrins (%)	
	Pyrethrin I	Pyrethrin II	Acid	Gnadinger-
Sample no.	(%)	(%)	method	Corl
B.	0·21	0·42	0·63	0·65
E.M. 2	0·45	0·60	1·05	1·06
E.M. 3	0·51	0·44	0·95	1·03
E.M. 6	0·40	0·58	0·98	0·94
E.M. 7	0·36	0·87	1·23	1·16
D.L.†	0·24	0·32	0·56	0·73
F. 11†	1·32	0·86	2·18	2·30
Sw.†	1·13	1·02	2·15	1·98
42*† (1)	0·20	0·24	0·44	0·40
(2)	0·20	0·22	0·42	—
82* (1)	0·38	0·60	0·98	0·92
(2)	0·39	0·60	0·99	—
G. 5 (1)	0·77	0·76	1·53	1·49
(2)	0·77	0·73	1·50	—
(3)	0·74	0·73	1·47	—
L.M.† (1)	0·67	0·87	1·54	1·51
(2)	0·74	0·84	1·58	1·47
(3)	0·66	0·99	1·65	—

* These samples were received from Messrs Gnadinger and Corl, who permit us to give their values: Sample 42, 0·39, 0·39; sample 82, 0·97, 0·97, 0·97, 0·94, in terms of total pyrethrins per cent.

† The ferricyanide Method A, to be described later (p. 125), gave results as follows: D.L. 0·70, F. 11, 1·93, Sw. 2·05, No. 42, 0·40, and L.M. 1·58, in terms of total pyrethrins per cent.

These samples, with the exception of Nos. 42, 82, and D.L., were all grown upon experimental plots from selected seed. They were known to be genuine *Chrysanthemum cinerariaefolium*, and were harvested and dried with the greatest care. For purposes of comparison, there are given also a few of the values obtained by the ferricyanide method, to be described later (p. 125). Four of the samples, 42, 82, L.M. and Sw., were

also tested by a biological method, in order to ascertain if there were concordance between the analytical data and the insecticidal values (p. 122). With one exception (D.L.) there cannot be said to be any very serious discrepancies between the results obtained by the different methods; the differences shown would scarcely be susceptible of detection by biological means. The sample D.L., in which the largest percentage discrepancy occurs between the results obtained by the acid method and the two others, was a commercial sample and seemed to us exceptional. It possessed an odour different from that of genuine pyrethrum.

In using the Gnadinger and Corl method we have introduced one minor modification, in that we have put the dextrose standard through exactly the same processes as the pyrethrum samples, except that of extraction with petroleum ether; thus the presence of any reducing matter in the alcohol used, although very small in any case, has not called for any correction. As thus applied, the Gnadinger and Corl method has proved of considerable value to us in checking samples of flowers from our experimental plots, which gave results which were unexpectedly high. In genuine samples, in the analysis of which great care has been exercised, we have so far had no serious discrepancy over a range of pyrethrin content of 0·40 per cent. to more than 2 per cent. Our experience of commercial samples has not been sufficiently great for us to say how great a concordance could be obtained in their case. Adulteration or fermentation due to inadequate drying or bad storage would possibly lead to discrepant results.

Errors due to "shaking-out" of sample.

During the course of the above investigations, we have noticed changes in the pyrethrin content of individual samples after being kept in tightly corked tubes for some months. This we have traced to a gradual "shaking-out" of the samples. In order to avoid formation of a hard cake during the petroleum ether extraction, with subsequent incomplete exhaustion, the samples were not ground too finely, approximately only 26 per cent. passing through a 100-mesh sieve. It was noticed that this finer fraction tended to separate out towards the top of the tube as the sample was kept, and was, moreover, very high in pyrethrin content. The fine fractions of the sample apparently consist of the more brittle parts of the flowers, namely the achenes, which, as Gnadinger and Corl[2] have shown, contain approximately 90 per cent. of the total pyrethrins of the flower heads. The fine powder obtained by

passing a normally ground sample through a 100-mesh sieve was found to contain 3·3 per cent. of total pyrethrins when tested by reduction of ferricyanide (Method A, p. 125), the residue on the sieve having a content of 1·30 per cent. The pyrethrin content calculated from the weights of the fractions and the determined content of each, agreed exactly with the figure obtained by analysis of the well-mixed samples (1·85 per cent.). It will be seen that a slightly irregular distribution of the more finely ground fractions of samples, such as we have been using, may lead to widely differing figures upon analysis. In order to avoid the possibility of erroneous results due to this factor, we suggest the samples be ground to an impalpable powder, and mixed with ignited sand prior to extraction with petroleum ether.

Insecticidal Tests.

The direct correlation of the analytical results with the insecticidal properties of samples showing widely ranging pyrethrin contents was realised as being of importance. We chose two commercial samples sent to us from the United States (Nos. 42 and 82), and two samples grown upon half-acre experimental plots and harvested in July 1930 and labelled L.M. and Sw. The analytical results are given in Table III, the samples showing total pyrethrin contents of 0·40 to 2 per cent. Each was extracted with absolute alcohol in the cold, the 10 per cent. extract was then diluted with saponin solution of 0·5 per cent. concentration, and the dilutions shown in Table III used for spraying.

The insects used were *Aphis rumicis*, and the trials were carried out in the way and with the apparatus already described[7]. Random samples were not taken of the whole of the insects available, but the adult apterous females were selected with considerable care and, for every concentration tested, ten insects were used. Repetitions were carried out on the same day at certain of the concentrations. After spraying, the insects, without further handling, were placed in petri dishes near fresh bean foliage and examined each day for 3 days. Each test was given a number, and the examination carried out and the result expressed in ignorance of the actual concentration to which it referred. The insects were put into four categories: (1) those not affected, N; (2) those somewhat affected but able to walk, S; (3) the moribund, i.e. those able to move appendages, M; (4) those apparently dead, D. Each individual insect was carefully examined each day to ascertain the extent of the toxic effect produced. The observations were not carried out later than

the third day after spraying, as the insects in the controls were showing signs of failure on that day; these few failures appear to have been generally due to the insects involved not having settled down on the foliage provided. The tests were carried out with concentrations ranging from those giving 100 per cent. moribund and dead insects to those in which the mortality was nil. The data should be inspected as a whole but, for purpose of ready comparison, we have given the percentage value of the moribund and dead taken together, and in addition have awarded marks by the addition of the percentage number of dead to half the percentage of moribund and one-quarter of the percentage of the slightly affected. By this latter method the grading effect is more definitely indicated by a single figure than by the former, but the toxic action is probably under-valued.

Table III. *Toxicities of pyrethrum samples to* Aphis rumicis.

Marks $= [S/4 + M/2 + D]$.

Sample no.	% of flowers	Average % of insects taken					
		N	S	M	D	M + D	Marks
42	1·0	—	—	30	70	100	85
	0·75	—	—	40	60	100	80
	0·50	10	20	25	45	70	62½
	0·35	25	25	30	20	50	41
	0·20	65	20	10	5	15	15
	0·10	90	10	—	—	0	0
	0·075	100	—	—	—	0	0
82	0·50	—	—	—	100	100	100
	0·35	—	—	5	95	100	97½
	0·20	—	—	15	85	100	92½
	0·10	42*	26	26	6	32	24
	0·075	50	40	10	—	10	15
	0·05	100	—	—	—	0	0
L.M.	0·20	—	—	—	100	100	100
	0·10	—	—	15	85	100	92½
	0·075	—	15	30	55	85	74
	0·05	20	50	10	20	30	37½
	0·025	45	25	10	20	30	31
	0·01	100	—	—	—	0	0
Sw.	0·35	—	—	—	100	100	100
	0·20	—	—	5	95	100	97½
	0·10	—	—	10	90	100	95
	0·075	—	—	25	75	100	87½
	0·05	—	15	25	60	85	76½
	0·025	45	20	25	10	35	27½
	0·10	90	5	5	—	5	4
	0·005	100	—	—	—	0	0
Controls:							
Saponin	0·5 %	95	—	—	5	5	5
Saponin-alcohol		90	3	3	3	6	4½

* Nine insects sprayed in one test.

The number of insects used was not sufficiently large to state with precision the concentration giving approximately 50 per cent. of moribund and dead (the best for purposes of comparison), and although we are unable to state whether the toxicity differences found between samples labelled Sw. and L.M. are completely significant, the insects were of sufficiently good quality and the data sound enough to indicate that the toxicities run in the same order as the pyrethrin contents, namely:

$$\text{Sw.} > \text{L.M.} > \text{No. 82} > \text{No. 42.}$$

We should tentatively deduce that the 50 per cent. mortality points would be at the following concentrations in terms of the percentage weight in terms of the flowers: Sw. between 0·025 and 0·05 per cent.; L.M. between 0·05 and 0·075; No. 82 between 0·1 and 0·2; No. 42 between 0·35 and 0·5, but in each case rather nearer the lower value. The total pyrethrin contents in percentages run: Sw. 1·98–2·15; L.M. 1·5–1·65; No. 82, 0·92–0·99; No. 42, 0·39–0·44; the order of the percentage contents of pyrethrin I was: Sw. 1·13; L.M. 0·66–0·74; No. 82, 0·39; No. 42, 0·20. There is, therefore, a fairly good concordance between toxicity trials and the analytical tests, but the data cannot be used for determining whether the pyrethrin I content alone is adequate for determining the value of a sample.

Close examination of the data shows that sample No. 42 is not quite as toxic as the analytical data would lead us to expect. There are some grounds for suspecting that, in this case, there has been some loss of toxicity, as the sample (Dalmatian) was derived from the harvest of the year 1926 and was much older than the others tested. If this loss of toxicity be actual, it points to the conclusion that none of the analytical methods available are capable of completely ascertaining its magnitude, and that for this purpose further investigation is required.

RAPID METHOD OF EVALUATION OF PYRETHRUM EMPLOYING SMALL QUANTITIES OF MATERIAL.

We have shown above in some detail that, for samples containing high contents of the pyrethrins, some little difficulty may be experienced in the analysis, and we have suggested certain slight modifications to meet these causes, but it was felt that a rapid method of evaluation, employing small quantities of the material, would be of value in providing an index to the amount of sample to be taken for subsequent critical evaluation by the acid method. Furthermore, the possession of

a method of estimating the poisons in a single flower head was regarded as desirable before the physiological and genetical relationships of the pyrethrum plant could be satisfactorily investigated.

The valuable method suggested by Gnadinger and Corl is based upon the reducing properties of the pyrethrins to copper alkaline tartrate solutions. It requires, however, too great a weight of material to be applicable to the analysis of minute amounts of material. During the course of this work we attempted to adapt the method devised by Schaffer and Hartmann for the estimation of small amounts of copper-reducing sugars, and although a certain degree of success was achieved, the titration end-point proved rather unsatisfactory, and we were led to explore other means of analysis. An adaptation of the Hagedorn and Jensen technique as used for the estimation of blood sugar(3) was then investigated, and this method was found to be sufficiently sensitive to detect, quantitatively, small amounts of pyrethrins. Partial reduction of a standard alkaline potassium ferricyanide solution is effected by means of the ketone group of the pyrethrolone fraction of the pyrethrin molecule, the degree of reduction being given by the estimation of the amount of ferricyanide present before and after the reaction. This is effected by liberating the iodine equivalent of the ferricyanide, and titrating with standard thiosulphate solution. Graphs have been constructed giving the relationship between the amount of ferricyanide reduced, as expressed in c.c. of $N/200$ thiosulphate, and the mg. of pyrethrins I and II in an aliquot portion of the final pyrethrin extract. Experimental methods have been worked out to enable the estimation of the poisons in:

(a) 0·5 gm. of powdered material.

(b) A single flower head (approx. 0·1 gm. material).

These will be referred to as Methods A and B respectively. It is perhaps necessary to point out that considerable care and some practice in the technique are required for the satisfactory application of both these methods.

Method A.

The following solutions are required, employing in all cases A.R. quality chemicals:

1. Alkaline ferricyanide solution:

Potassium ferricyanide	1·649 gm.	
Anhydrous sodium carbonate	28·6	,,

made up to 1 litre with distilled water.

2. Ferrocyanide precipitant:

Potassium iodide	5 gm.
Hydrated zinc sulphate	10 „
Sodium chloride	50 „

in 200 c.c. of solution.

3. Acetic acid. 3 c.c. glacial acid in 100 c.c. of solution.

4. Hydrated zinc sulphate 1·25 gm. in 250 c.c. of solution.

5. $N/10$ sodium hydroxide.

6. Starch solution. 1 gm. soluble starch in 100 c.c. of solution and saturated with 20 gm. sodium chloride.

7. Aldehyde-free absolute alcohol.

> Absolute alcohol, containing 5 gm. *m*-phenylene diamine hydrochloride per litre, is allowed to stand 24 hours with frequent shaking, refluxed for 8 hours and then distilled, being kept in small well-filled stoppered bottles in a dark place, as suggested by Gnadinger and Corl[1].

8. $N/200$ thiosulphate. 1·243 gm. per litre, using boiled-out distilled water, and protected with a soda-lime tube. The thiosulphate solution is most readily standardised by titration of a $N/200$ solution of potassium iodate, prepared by weighing exactly 0·1784 gm. of the dried solid (A.R. quality) and making up to a volume of 1 litre in a standardised volumetric flask. 10·0 c.c. are accurately delivered into a small conical flask by means of a carefully calibrated pipette, employing a definite drainage period. Addition of 5 c.c. of 2 per cent. potassium iodide solution, followed by 3·0 c.c. of 3 per cent. acetic acid solution, liberates the iodine equivalent of the potassium iodate, which is then titrated with the thiosulphate solution.

Preparation of pyrethrin extract. 0·5 gm. of material is extracted with petroleum ether (B.P. below 40° C.) in a Soxhlet apparatus, and the solvent removed by gentle warming, in a current of CO_2, final traces being removed in a vacuum desiccator. The residue is extracted on a boiling water bath with five successive portions of 4 c.c. each of aldehyde-free absolute alcohol. To the hot solution 1 c.c. of $N/10$ NaOH, and then 4 c.c. of dilute zinc sulphate are added, the solutions mixed, and warmed on the water bath for a few minutes, precipitation of proteins being thus effected. The solution is cooled to 20° C., made up to 25 c.c. with aldehyde-free alcohol, shaken and allowed to stand. The final protein-free extract is obtained by filtering through a small Whatman No. 1 filter paper. For the estimation, 2 c.c. of extract (delivered from a fine nozzle and standardised pipette) are heated for a definite time in a boiling

water bath with 10·0 c.c. of alkaline ferricyanide solution A (accurately delivered), in a Folin tube[1]. The solution is then cooled, washed into a small conical flask, and excess of ferrocyanide precipitant immediately added. The iodine equivalent of the remaining ferricyanide is liberated
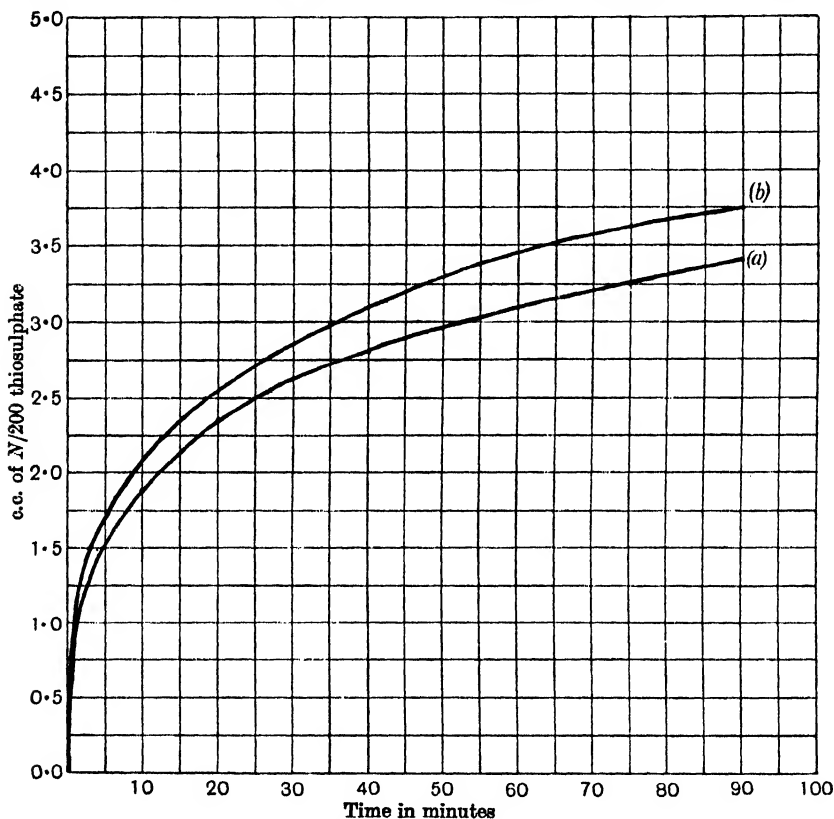
Fig. 1. Graphs *a, b*

by addition of 10 c.c. of 3 per cent. acetic acid, and, by means of a micro-burette, titrated with thiosulphate, using starch as indicator towards the end of the reaction.

A blank determination on 10 c.c. of ferricyanide is carried out, using 2 c.c. of 80 per cent. alcohol. We have preferred to prepare the alcohol in a way exactly similar to the preparation of the pyrethrin extract, by

[1] After experimenting with test-tubes of various dimensions we found the Folin bulb and tube modified by Gnadinger and Corl(1) a suitable vessel in which to carry out the reduction. The dimensions are: bulb to contain 15·5 c.c. with narrow portion of tube 4 cm. long, and with an internal diameter of 6–7 mm.

addition of sodium hydroxide and zinc sulphate to 20 c.c. followed by heating, and filtering after making up to 25 c.c. We have, however, found no significant differences between blank determination on 10 c.c. of reagent when using this treated alcohol, and that prepared directly from aldehyde-free absolute alcohol.

From the difference in the thiosulphate titrations the amount of pyrethrins in 2 c.c. of the filtrate may be read directly from graph A (Fig. 2). The pyrethrins in 25 c.c. of the extract and, therefore, in 0·5 gm. of material used, can thus be readily obtained.

Period of heating necessary for the oxidation of the pyrethrins. An extract of pyrethrum, containing a known amount of poisons, was obtained, and portions of 2 c.c. each heated in a boiling water bath for intervals of 10, 20, 30 minutes, etc. up to $1\frac{1}{2}$ hours. The amount of reduction was estimated in each case and correlated with time of heating. The results are expressed in Fig. 1 (*a*). It will be seen that most of the pyrethrin oxidation is effected in the first 45 minutes, there being after this a further slight reduction, approximately constant in amount over further equal intervals of time. This effect was still observed after heating for a period of 2–3 hours, and was not reduced by precipitation of resin acids as their barium salts, together with proteins in the preparation of the extract, by employing barium hydroxide and zinc sulphate. It is probably due to slight interaction between the ferricyanide and alcohol upon prolonged heating. The convenient period of 45 minutes was, therefore, taken as being the minimum time for oxidation of the pyrethrins under these conditions.

Standardisation of graph. A sample of pyrethrum was analysed by the acid and Gnadinger and Corl methods; the results obtained were as follows:

Acid method:
(1) Pyrethrin I, 0·77 per cent.; pyrethrin II, 0·76 per cent.
     Total pyrethrins, 1·53 per cent.
(2) Pyrethrin I, 0·74 per cent.; pyrethrin II, 0·73 per cent.
     Total pyrethrins, 1·47 per cent.

Gnadinger and Corl method:
(1) Total pyrethrins, 1·49 per cent.

The sample had the advantage of being very rich and of containing the two active principles in equal proportions; a mean value of 1·5 per cent. total pyrethrins was taken. It was extracted with petroleum ether (B.P. below 40° C.) and freed from proteins in the way outlined above. A number of dilutions of known strength were prepared, and 2 c.c.

portions of each heated with 10 c.c. of alkaline ferricyanide for 45 minutes in a boiling water bath. The amount of reduction was estimated in each case, and, expressed in terms of $N/200$ thiosulphate, was plotted against the mg. of pyrethrins contained in 2 c.c. of each extract (Graph A, Fig. 2). The amounts of pyrethrins per 2 c.c. of extract ranged from 0·16 mg.
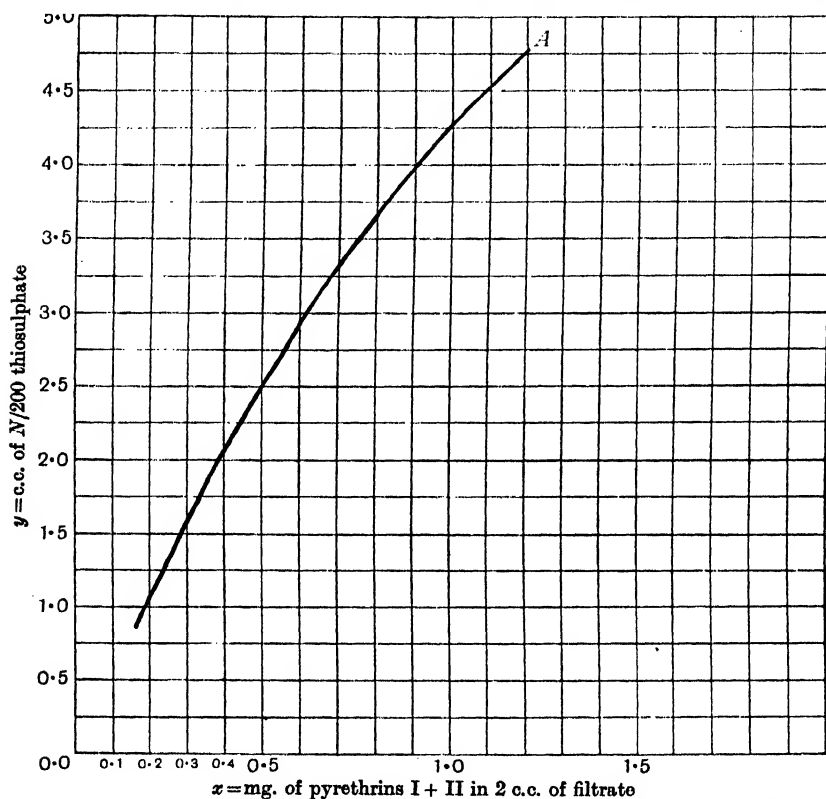
Fig. 2. Graph A. 0·5 gm. of material; equation $y = -0·067 + 5·969x - 1·616x^2$

to 1·2 mg., giving a range of values for the pyrethrin content, when expressed on 0·5 gm. of material, of from 0·40 to 3 per cent. The points as determined experimentally were found to be close to the parabolic curve as expressed by the equation

$$y = -0·067 + 5·969x - 1·616x^2,$$

where

y = c.c. of $N/200$ thiosulphate equivalent to the reduction,

x = mg. of pyrethrins I and II in 2 c.c. of filtrate.

The values for x and y used in computing the graph are given below:

When	$x = 0.16$	$y = 0.864$
	0.30	1.578
	0.40	2.062
	0.48	2.426
	0.60	2.932
	0.69	3.282
	0.86	3.871
	1.00	4.286
	1.20	4.769

Results obtained by using this method are summarised in Table IV, together with the analyses carried out by the acid method.

Table IV. *Analytical results by Method A.*

Sample no.	Acid method			Total pyrethrins (%)	
	Amount taken (gm.)	Pyrethrin I (%)	Pyrethrin II (%)	Acid method	Ferricyanide method A
42	10.0	0.20	0.24	0.44	0.40
E. 12 buttons	5.0	0.23	0.30	0.53	0.55
E. 12 ¾ open	5.0	0.32	0.37	0.69	0.69
E. 8 (1)	5.0	0.30	0.38	0.68	0.78*
(2)	2.5	0.53	0.46	0.99	0.90
E. 6	5.0	0.29	0.45	0.74	0.80
D.L. (1)	5.0	0.24	0.32	0.56	0.70
(2)	5.0	0.23	0.36	0.59	—
E. 5 (1)	5.0	0.45	0.46	0.91	0.90
(2)	5.0	0.44	0.51	0.95	—
F. 12	5.0	0.42	0.57	0.99	1.00
F. 10	2.5	0.39	0.58	0.97	1.12
E. 10 (1)	5.0	0.44	0.56	1.00	1.08
(2)	2.5	0.47	0.58	1.05	—
F. 2	2.5	0.60	0.56	1.16	1.10
E. 3	2.5	0.50	0.62	1.12	1.25
E. 7	5.0	0.56	0.61	1.17	1.25
E. 4	2.5	0.39	0.79	1.18	1.28
E. 9	5.0	0.62	0.56	1.18	1.30
F. 4	2.5	0.70	0.69	1.39	1.35
F. 3	5.0	0.56	0.65	1.21	1.40
E. 11 (1)	5.0	0.90	0.70	1.60	1.65
(2)	2.5	0.95	0.77	1.72	—
D. 12	2.5	0.41	0.80	1.21	1.40
D. 11	2.5	0.23	1.18	1.41	1.63
F. 8	2.5	0.78	0.95	1.73	1.73
G. 8	2.5	0.95	0.95	1.90	2.10
F. 11	2.5	1.32	0.86	2.18	1.93
L.M. (1)	5.0	0.67	0.87	1.54	1.58
(2)	5.0	0.74	0.84	1.58	1.58
(3)	2.5	0.66	0.99	1.65	—
Sw.	5.0	1.13	1.02	2.15	2.05

* The discrepancies noted in E, 8 were largely due to the shaking-out of the sample (p. 121).

It will be seen that fairly good agreements hold for analyses over a range of pyrethrin contents extending from 0·40 to 2·0 per cent., particularly close agreements being observed in the sample L.M., when two observers independently obtained 1·58 per cent., a value agreeing with the average figure by the acid method. The greatest observed discrepancies occur in the F. 11, D. 11 and D.L. samples. In the first of these, however, the pyrethrin content was remarkably high, while the D.L. sample was of unknown commercial origin, and did not possess the odour characteristic of pyrethrum flowers. In this case, however, the result by the ferricyanide method is seen to be in excess of the acid method figure, but agrees with the value given by the Gnadinger and Corl method. We should state that the method has not been primarily devised for the detection of adulteration, but for the evaluation of unadulterated samples of known origin. Where approximate rapid analyses with small quantities of material were required, the method has been of value. In its present form, however, it does not appear applicable to the evaluation of flower heads in the very early stages of development (minute buds). In some of these cases we have recorded results considerably in excess of those given by the acid method. The hypothesis that in some cases reducing bodies, similar to pyrethrolone but not linked with the pyrethrin acids, may exist in the plant has already been tentatively suggested, and indications that this may be the case are particularly strong when dealing with flowers in the early stages of development.

Method B.

Evaluation of single flower heads. Individual flower heads selected at random from an air-dried English-grown sample were weighed, the results being as follows: 0·16, 0·12, 0·14, 0·09, 0·10, 0·13, 0·12 gm.

It, therefore, seemed useful to attempt to adapt the method to estimate the active principles in weights round about 0·1 gm. The only modification in the solutions used is in the ferricyanide reagent.

1. Alkaline ferricyanide solution B:

    3·30 gm. potassium ferricyanide.

    57·20 gm. anhydrous sodium carbonate in 1 litre of solution.

The other reagents used are as in Method A, where 0·5 gm. of material is taken. In the actual analysis, after precipitation of the protein as in Method A, 10 c.c. of the protein-free extract of the pyrethrins are taken, together with 5 c.c. of the alkaline ferricyanide solution B. In this case, heating is carried out in a bath, fitted with a stirrer, and controlled at 78° C. As with Method A, a period of heating of 45 minutes

is employed, since it was observed that, under these conditions, most of the oxidation is effected in the first 45 minutes (see Graph *b*, Fig. 1). A lagging effect, similar to that observed in standardising the time of heating in Method A, was also observed.

Blank determinations on 5 c.c. of the ferricyanide reagent are carried out as before, using 10 c.c. of aldehyde-free alcohol, treated as described in Method A. In Graph B (Fig. 3), standardised in a similar way to Graph A, is recorded correlation data between the amount of reduction, expressed in c.c. of $N/200$ thiosulphate, and the mg. of pyrethrins I and II in 10 c.c. of protein-free extract. From the differences in the readings observed between the blank and the test titrations a simple calculation gives the total pyrethrin content of the sample.

The experimental values were found to fit closely the curve expressed by the equation

$$y = -0.125 + 4.143x - 0.318x^2,$$

where　　$y =$ c.c. of $N/200$ thiosulphate,

　　　　$x =$ mg. of pyrethrins I and II in 10 c.c. of extract.

The values used in constructing the curve are as follows:

When　　　　　　$x = 0.15$　　　　　　$y = 0.489$
　　　　　　　　　　0.30　　　　　　　　1.089
　　　　　　　　　　0.44　　　　　　　　1.636
　　　　　　　　　　0.538　　　　　　　2.012
　　　　　　　　　　0.60　　　　　　　　2.246
　　　　　　　　　　0.867　　　　　　　3.228
　　　　　　　　　　1.20　　　　　　　　4.389

Results obtained by analysis of 0.1 gm. of sample, together with results given by the acid method, are summarised in Table V.

Table V. *Analytical results by Method B.*

Sample no.	Acid method			Total pyrethrins (%)	
	Amount taken (gm.)	Pyrethrin I (%)	Pyrethrin II (%)	Acid method	Ferricyanide method B
42	10.0	0.20	0.24	0.44	0.50
82	5.0	0.38	0.60	0.98	1.00
E. 8	5.0	0.30	0.38	0.68	0.70
E. 6	5.0	0.29	0.45	0.74	0.73
F. 12	5.0	0.42	0.57	0.99	0.98
E. 3	2.5	0.50	0.60	1.10	1.08
E. 7	5.0	0.56	0.61	1.17	1.10
L.M. (1)	5.0	0.67	0.87	1.54	1.60
(2)	5.0	0.74	0.84	1.58	—
(3)	2.5	0.66	0.99	1.65	—
F. 11	2.5	1.32	0.86	2.18	1.85

The variation in pyrethrin content in flower heads from one plant, at different stages of development, was next investigated. Individual flower heads, showing successive stages from the half open to the fully overblown conditions, were taken. The stalks were completely removed, the heads finely ground, and dried in an oven at 100° C. for 2 hours,
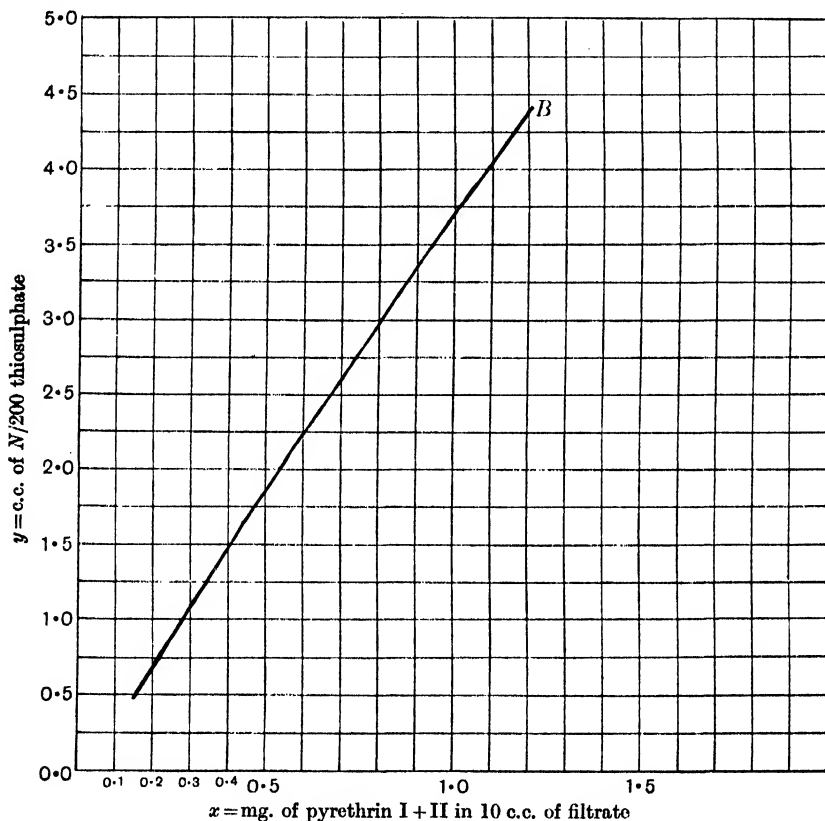
Fig. 3. Graph B. 0·1 gm. of material; equation $y = -0·125 + 4·143x - 0·318x^2$

weighed accurately, and extracted with petroleum ether after grinding with sand. The residues were taken up with aldehyde-free alcohol, the proteins precipitated, and estimation of the active principles carried out. The results, given in Table VI, were expressed both as mg. of total pyrethrins per flower head and as their percentage content.

These results lend support to the observation to be recorded elsewhere that a significant drop in the pyrethrin content occurs soon after

the final stage of maturity has been reached, *i.e.* when all the disc florets are in an open state. This fall in poison content appears to commence with the first appearance of discoloration of the petals. It will be seen from Table VI that, when expressed both as weight per flower head, and as percentage, the maximum content coincides with the opening of all the disc florets, but, in contradistinction with the fall in percentage pyrethrin content between the fully opened and overblown flower heads, if the pyrethrins are expressed as mg. per head the difference is scarcely significant.

Table VI. *Relationship between stage of development of flowers and pyrethrin content.*

Stage of development	Weight of flower head (gm.)	Total vol. of extract (c.c.)	Pyrethrins per head (mg.)	Pyrethrins per head (%)
Half open	0·0604	25	0·87	1·44
Three-quarters open	0·0848	25	1·18	1·39
First row disc florets open	0·1070	25	1·60	1·50
Half disc florets open	0·1388	25	2·16	1·56
All disc florets open	0·1918	50	3·15	1·64
Overblown	0·2805	50	2·80	1·00

We have found that some considerable confusion exists in the terminology attached to the different stages of development of pyrethrum flower heads. In order to standardise this as far as possible, we have taken as "half open flowers" those showing a tubular arrangement of the petals. Flowers showing one or more of the outer rings of disc florets open we have termed "fully open," while intermediate stages in which the petals are seen expanding, or fully expanded but with no disc florets open, we have designated "three-quarters open." Individual flower heads showing these various stages were taken and photographed.

In Plate I (*a*) and (*b*), from 1 to 5, are shown respectively half open flowers, three-quarters open flowers and flowers showing the first row of disc florets open, approximately half the disc florets open, and practically all the disc florets open. Thus under our terminology, flowers 3, 4 and 5 are fully open. It is seen from Table VI that the "fully open" flowers all have the pyrethrin contents of the same order, *i.e.* in the region of 1·5 per cent.

We desire to express our indebtedness to Miss A. M. Webster of the Statistical Department for determining the equations of Graphs A and B.

(*a*)
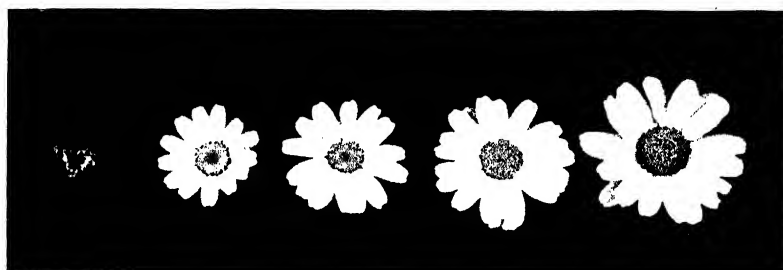
(*b*)

Summary.

1. The analytical methods of Tattersfield, Hobson and Gimingham[6] and Gnadinger and Corl[1] for the determination of the pyrethrins in pyrethrum flowers are compared, and certain modifications in technique suggested.

2. Good concordances have been obtained between analytical data and insecticidal tests employing *Aphis rumicis*.

3. A new method for the rapid and approximate evaluation of unadulterated samples, employing small quantities of material, is described.

4. Observations on the pyrethrin content of individual flowers in the various stages of development are recorded, making use of a modification of the method indicated under (3).

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THE AVERAGE AGES OF COWS AND BULLS IN SIX BREEDS OF CATTLE.

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I. AVERAGE AGE OF COWS IN PEDIGREE HERDS.

ACCORDING to Sanders (1928) and to Kay and M'Candlish (1929), who substantially agree with each other as well as with investigators in other countries, it is known that a dairy cow reaches her maximum production of milk around the eighth year of her life—that is to say, between the ages of 7 and 8. The yield of a heifer calving at the customary age of 2 years (*i.e.* from 24–36 months after birth) is, roughly speaking, only four-fifths of what her yield as a mature cow is likely to be. The yield does not decline greatly after maturity till the twelfth year is reached. For the purpose of the present argument it may be taken that, as a general rule, the period of the life of a dairy cow during which she is an economic producer of milk extends for 10 years, from $2\frac{1}{2}$ to $12\frac{1}{2}$ years of age, and that maximum production is reached at roughly midway, or at the age of $7\frac{1}{2}$. (M'Candlish (1928) has shown that, with Ayrshire cows, the best age for first calving is $2\frac{1}{2}$ years.)

The product of the beef cow is a calf born and reared annually with, in many cases, an additional milk supply for the nourishment of other calves. There are no data to show the age at which a beef cow reaches maturity, or when the average cow ceases to be able to produce a calf annually. Perhaps $7\frac{1}{2}$ years may be adopted as the mature figure, since it is reasonable to assume that in beef cattle the yield of milk will also reach its maximum at that age when the cow should be either carrying or nursing her sixth calf. Again, $12\frac{1}{2}$ years might be selected—though somewhat arbitrarily—as the age at which a beef cow ceases to be an economic producer.

If every cow were a good producer, if there were no disease or accidents, then the ideal situation would arise in our herds of having an equal number of cows of each age in each well-established herd. Working on the above assumption that the productive life of a cow lasts for 10 years (*i.e.* $12\frac{1}{2}$ years of age) then the average age of the cows should be $7\frac{1}{4}$ years and, since at any age there would be found 10 per cent. of

the cows in the herd, the annual replacement number—that is to say, the number of heifers drafted into the herd annually—should be 10 per cent.

It may be thought that this standard is too high and the curve too regular. It is taken in the present instance as a foundation for argument rather than as an accepted fact. It is a standard approaching the ideal, but not quite. It is ideal in that it assumes that there are no losses amongst the dairy cows before they cease to be economical producers, and also in that it assumes that the average cow will produce a calf every 12 months. But this may be balanced by the fact that, since many cows are economical producers for several years after their twelfth birthday, 12½ years is probably too low to set as the ideal. In the following study cows were found producing calves at every age class up to 21 years. In any case the figures that the average age at calving of the cows in an ideal herd should be 7½ years and that the annual loss of breeding cows which has to be replaced should amount to 10 per cent. are taken as a standard by which existing facts can be compared.

Lawson (1929), working with 1267 dairy cows in West Sussex, has shown the average life of a cow in a dairy herd to be 2½ years. If the average age at which a cow calves for the first time be taken as 2¼ years, the average age of the cows studied would be about 5 years. Lawson has also stated that the annual replacement figure was 31 per cent. of the total number of cows, but this included 6 per cent. "sold to dealers," and made the true replacement figure somewhat more than 25 per cent.

Roberts (1929), working with 260 cows in North Wales, makes the annual replacement figure to be about 26 per cent. and, while he does not state the average age of the cows at time of calving, it may be deduced from his figures that this was around 5½ years.

These figures are in the main substantiated by Hunter-Smith (1929), Wyllie (1929) and Kay and M'Candlish (1929). The latter state that from their experience of the dairy herds in the south-west of Scotland the average length of life of the dairy cow is about 4 years. Allowing for the length of lactation of nearly a year, this, on the basis that the average cow calves for the first time at 30 months, brings the average age at calving of the cows which they studied to be about 5·75 years. Three American reports, "T.J.B." (1912), Gowen and Covell (1921), and Shepherd (1929), give the average productive life of dairy cows as from 5 to 9 years. In the first case, based on pedigree records, the methods employed were far from accurate. The other two results were not supported by data. The statement of the British Friesian Cattle Society that the average working life of 300 British Friesian cows yielding over

2000 gallons is about 7 years is based on a selected population. M'Candlish (1930) reviews the whole subject, particularly in relation to age at first calving.

The object of the present study, which was begun in 1926, was to gain information concerning the average age of cows in the various pedigree breeds more with a view to making a general reconnaissance of the position than in hope of getting positive information about details. The object was rather to find out whether there was room for improvement and, if so, how much. The primary intention was to obtain guidance as to whether further investigations of the problem would be fruitful from a strictly economic point of view.

The materials used were the herd books of six of the leading breeds of cattle in this country: Jersey, Ayrshire, British Friesian, Aberdeen-Angus, Hereford and Shorthorn. The Shorthorns were divided into Dairy Shorthorns and Beef Shorthorns. The Dairy Shorthorn together with the Jersey, Ayrshire and Friesian were classed as "Dairy Breeds," while the Beef Shorthorn was grouped with the Aberdeen-Angus and Hereford as "Beef Breeds."

For each of these breeds a random sample of the births in the year 1925 was taken. This was done by selecting the entries at the top of every page (or of every alternate page) of the respective herd books. The date of the birth of each parent was then traced back in the previous volumes.

Table I shows the number of births thus traced in each of the herd books.

Table I. *Number of births traced.*

	Bull calves	Heifer calves	Total
Jersey	155	226	381
Ayrshire	95	236	331
British Friesian	120	241	361
Shorthorns	214	395	609
Hereford	190	238	428
Aberdeen-Angus	257	251	508
Total: all breeds	1031	1587	2618

Herd book records must frequently be accepted with considerable reserve but, in this case, there is not likely to be any great proportion of error, and such as there may be would not compare with the error likely to be obtained where registration of colour, etc., is concerned.

Table II shows the average age of the cows at time of calving in the four dairy breeds. Since the numbers of each breed are comparatively

small, and in order to avoid misrepresentation, the breeds are not named but numbered.

Table II. *Averages and standard deviations of ages of cows at calving in four breeds of dairy cattle.*

	Average age at time of calving (years)	Probable error	Standard deviation
No. 1 breed	6·381	±0·10	2·611
No. 2 „	5·283	±0·09	2·619
No. 3 „	5·024	±0·09	2·741
No. 4 „	5·463	±0·10	2·951
All dairy breeds	5·485	±0·05	2·578

One cow was found aged 15 years at calving, while ten calved at 14 years of age: 2·4 per cent. of the cows calved at over 12 years of age. In passing it might also be noted that 29·2 per cent. of the dams of heifers calved at 3 years or younger, while only 24·8 per cent. of the dams of bulls calved at that age. The difference is probably due to the fact that there is a tendency to register bull calves in dairy breeds on the production of their dams, the milk records of which are not available till they are at least 3 years of age.

The figures obtained here substantiate the work of previous investigators that the average productive life of a cow in a dairy herd is about 3½ years, and that the annual replacement figure is therefore roughly 29 per cent.

Turning now to beef cattle, the details of which are set out in Table III, we find that there is a tendency for a slightly longer life by about 6 months.

Table III. *Averages and standard deviations of ages of cows at calving in three breeds of beef cattle.*

	Average age at time of calving (years)	Probable error	Standard deviation
No. 5 breed	6·339	±0·10	3·238
No. 6 „	6·018	±0·08	2·755
No. 7 „	5·276	±0·11	2·481
All beef cattle	6·022	±0·05	2·676

With the exception of one breed, there is no difference in the number of bulls recorded as compared with heifers from cows of any particular age. Since, in the exception, the difference is less than three times the probable error, it can be ignored. The cows with the greatest ages were

found in No. 5 breed, where births were recorded from one cow aged 21 years, one aged 20, one aged 19 and four aged 17. In the other two breeds the greatest ages were respectively recorded as 18 years (two cases) and 14 years (two cases). 3·2 per cent. of the calves recorded were from cows of over 12 years of age, as compared to 2·4 in the dairy breeds.

In the beef breeds the average productive life of a cow is about 4 years, which gives an annual replacement figure of about 25 per cent.

The results of Tables II and III are combined in Table IV:

Table IV. *Average and standard deviation of ages of cows at calving in six British breeds of cattle.*

Average age at time of calving (years)	Probable error	Standard deviation
5·722	±0·04	2·816

Let 2½ years be taken as the average age at which heifers drop their first calf. (In some breeds this is higher, in others lower. Possibly the actual figure should be somewhat higher, but it is better to err on the conservative side.) Then the length of the productive life of the cow is 5·72 less 2·5 years which makes 3·22 years. But since this is the average age at time of calving, it may be presumed that the cow remains in the herd for anything up to another year. Let 6 months be taken as the average length of productive life of a cow in a pedigree herd after her final calving. This, added to 3·22 years, gives a figure of roughly 3·7 years as the average length of total productive life, and this in turn means that there is an annual replacement of roughly 27 per cent. of the cows in the six pedigree breeds studied.

Whether this figure of an annual replacement of about 27 per cent. of productive cows can be taken as typical of the cattle industry of Great Britain is open to question. As the figures relate to pedigree cattle, it might be assumed that fewer of the productive cows would be sold at the older ages to breeders who would not register their progeny, but against this should be placed the fact that, in theory at any rate, pedigree herds should be more closely culled than the ordinary "commercial" ones and that, therefore, there should be a lower average age of cows in pedigree than in other herds. It is extremely doubtful, however, whether our livestock industry has reached such an ideal position. At the moment many commercial breeders cull their animals to a greater extent than the pedigree breeders. For instance, whereas the commercial

man will instantly dispose of a "shy breeder," the pedigree man is more tempted to keep her on in hopes of getting another calf.

On the whole it can be taken that these figures are not far off the mark for the average of the cows in the United Kingdom, especially as they are substantially in accord with the results of other workers. Undoubtedly differences may be expected from district to district as they have been found to exist between breeds. But many of these differences may be due to other facts, such as age at first calving, etc., which are partly influenced by the breed and partly by the type of husbandry.

That an extension of the average age at calving and a lowering of the annual replacement of female stock will prove of financial benefit to the farmer is shown in Table V. For the purpose of this table the value of a heifer at the time at which she drops her first calf is taken as £30. Since she has also a "butcher" value of, say, £15, her net cost of production may be reckoned as £15. If she remains in the herd for only one lactation, this cost must be put against the value of her produce. The longer she remains in the herd as a producer the lower is this annual cost against her produce.

Table V. *Cost of cow chargeable against the value of the produce of that cow in respect of the length of her productive life, with additional figures for a herd of twenty cows showing annual replacement cost in respect of the annual replacement percentage.*

Annual replacement (%)	Average no. of calvings	Probable age in years at calving	"Butcher" value of cow £	Net cost per cow £ s.	Annual replacement cost per herd of 20 cows £
100	1	2¼	15	15 0	300
50	2	3¼	15	7 14	154
33·3	3	4¼	15	5 5	105
25	4	5¼	14	4 6	86
20	5	6¼	14	3 10	70
16·7	6	7¼	13	3 4	64
14·3	7	8¼	13	2 16	56
12·5	8	9¼	12	2 13	51
11·1	9	10¼	12	2 8	48
10	10	11¼	12	2 4	44

The gross value of a heifer at first calving is taken as £30. Compound interest is reckoned at 5 per cent. The values are calculated to the nearest shilling.

At the present moment in the six pedigree herds studied the annual replacement figure is certainly not less than 27 per cent. This means that a sum of roughly £4. 10s. must be placed as an initial cost against the produce of each lactation. And this is so only if the cow is a regular

producer each year. Should she fail to calve regularly, this cost will be considerably increased. Many cows do fail to calve regularly. However, let error be again upon the conservative side, and it is found that the annual replacement cost in a herd of twenty cows which calve regularly every year is £90.

If the ideal annual replacement figure of 10 per cent. were attained, the net amount per cow to be put as this charge against the produce of each lactation would, provided she had calved regularly, amount to just over £2, giving a net annual replacement cost of some £45 in a herd of twenty cows. This would effect a saving of £45 per annum on existing conditions. And even if the annual replacement percentage were reduced to a less extent, say to 20 per cent.—a figure which should prove practicable—the saving per cow compared to existing conditions would amount to about £1 for each lactation, or about £20 a year in a herd of twenty cows. In addition it must be remembered that if the herd consists of a large proportion of first calvers the output of milk is considerably less; the net cost quoted above does not take this into consideration, though allowance should be made. For instance, the reduction of the replacement percentage from 27 to 20 in a herd of twenty cows would increase production by about 300 gallons per year, which at 1s. per gallon amounts to £15 per annum. Thus the total economy effected by what is really quite a small improvement would amount to around £35 a year: this should have some effect on the profit and loss account.

It is outside the scope of this paper to analyse the reasons for this large annual replacement figure. Reference may, however, be made to the work of Lawson (1929) who, in computing the annual replacement of herds in West Sussex which amounted to 31 per cent., found this figure to be made up as follows:

	%
Died	6
Failure to breed	6
Rejected for abortion	1
Poor yielders	6
Sold to dealers	6
Loss of quarters	2·3
Johne's Disease	2·5
Tuberculosis	1·7
Total annual loss	31·5

Roberts (1929) found that the annual replacement figure was so high as to permit of no selection in the home-bred heifer calves of many herds: and that summer calvers were more subject to wastage than spring

calvers. He states that he found a higher rate of wear and tear in herds producing Grade A or certified milk. The experience of the present writers leads them to believe that, in herds which have been producing such milk for several years, the replacement percentage is lower than in other herds.

The object of this work was to reconnoitre the position, and the conclusion which may be arrived at is that, by prolonging the average productive life of our cows, a considerable economy in production can be effected, and that it is feasible to effect this economy. Further information is still required to show the reasons for the low average productive life and high annual replacement percentage. Concerning these, enquiries are at present being made both in England and Scotland. Only when detailed reasons are available for the large annual losses will it be possible to attack this problem in a rational manner; or, in other words, to concentrate our research upon those problems concerning which the present state of agriculture most demands its aid.

From the point of view of stock improvement it is essential that this annual replacement percentage should be considerably reduced for, as Roberts has pointed out, at its present figure it does not permit of adequate selection of the heifers which are drafted in annually to maintain the female stock of our herds. Thus, a lowering of the replacement percentage, besides having an immediate economic effect, would also have perhaps an even greater subsequent effect in the general improvement of the cattle of this country.

II. Average age of bulls in pedigree herds.

At first sight the average age of the cow at time of calving would appear to be of greater importance than the average age of the bull at the time of the birth of their progeny, since from this figure can be calculated the annual replacement percentage of cows in pedigree herds. But if it is remembered that the surest method of estimating the genetic value of a bull is by assessing the quality of his calves—especially in relation to the dams of those calves—it will be seen that information concerning the average age of bulls should throw some light on the methods adopted for the improvement of cattle in this country.

The data and methods employed to enquire into this problem were the same as those which provided the material for Part I of this paper. Taking the dairy breeds first, it was found that the average age of bulls at the time of the birth of their progeny was 3·687 years. The details are set out in Table VI.

Table VI. *Average and standard deviations of ages of bulls at the time of birth of their progeny in four breeds of dairy cattle.*

	Average age at birth of progeny (years)	Probable error	Standard deviation
No. 1 breed	3·879	±0·07	1·769
No. 2 ,,	3·975	±0·07	1·827
No. 3 ,,	3·257	±0·05	1·437
No. 4 ,,	3·678	±0·06	1·693
All dairy breeds	3·687	±0·03	1·763

Save for one breed, in which perhaps the bulls are used at a somewhat earlier age than in the others, there is no remarkable difference between the ages of the bulls. There is a slight but not significant tendency for the sires of bull calves to be somewhat younger than the sires of heifer calves.

That the beef breeds are again somewhat better than the dairy ones will be seen from Table VII.

Table VII. *Average and standard deviation of ages of bulls at the time of birth of their progeny in three breeds of beef cattle.*

	Average age at birth of progeny (years)	Probable error	Standard deviation
No. 5 breed	4·275	±0·06	2·136
No. 6 ,,	4·063	±0·07	2·192
No. 7 ,,	3·407	±0·08	1·717
All beef breeds	4·052	±0·04	2·09

There is a significant difference between No. 7 and the other two beef breeds, but it is difficult to see any specific reason for this. There is practically no difference between the percentages of heifer and bull calves by bulls of similar ages. The oldest beef bull noted was 20 years, while the oldest dairy bull was 15. 3 per cent. of the sires of the beef calves were over 9 years of age as compared to only 1·2 per cent. in the case of the dairy breeds.

The figure 3·8 years represents the average age of all the bulls studied at the birth of their progeny. But, since many bulls are dead by the time their progeny are born, a more accurate figure is obtained by reckoning to the time of conception of the progeny, which is 9 months earlier or 0·75 of a year. Thus the average age of bulls in use for all the

pedigree breeds studied is found to be roughly 3·1 years, while for the beef breeds this figure is 3·3, and for the dairy breeds it is 2·9.

Table VIII. *Averages and standard deviations of ages of bulls at the time of birth of their progeny in six British breeds of cattle.*

Average age at birth of progeny (years)	Probable error	Standard deviation
3·846	±0·03	1·943

It is not customary to use a bull before he is 12 months of age. Some breed societies refuse to register stock by bulls of less age. Nor can a bull be freely used till he is 18 months of age. Splitting the difference, let us take 15 months as the average age at which bulls are first used. This makes the average productive life of a pedigree bull 1·85 years. For the beef bulls it is 2·05 and for the dairy bulls 1·65 years. Owing to the fact that in many pedigree herds it is not unusual to use on the heifers a young bull before selling him, these figures may perhaps be not quite representative of commercial herds: but this in no way explains the paucity of breeding bulls above 3 years of age.

From this the annual replacement percentage of bulls in pedigree herds can be calculated. For all the breeds studied this is roughly 54 per cent. For the beef bulls the replacement percentage is about 49 per cent., while for the dairy bulls it is just over 60 per cent. In effect this means that, on the average, pedigree breeders with only one bull in their herd procure a new one every second year.

The average price of the yearling bull calf varies somewhat according to the breed. Let it be taken as £30. Since, however, the greater number of pedigree bull calves sold annually are purchased by non-pedigree breeders for the improvement of their stock, the average price quoted at the sales is not the same as the average price paid by established pedigree breeders. This price is probably at least £50, even after allowing for the "butcher" value of the bull. Thus, if the useful life of a bull extends to only 1·85 years, the annual amount which (together with his maintenance, etc.) must be charged up against the calves he produces is, on a very conservative estimate, at least £27 per annum; for the beef herds this figure is more than £24, while for the dairy herds it is over £30 a year. Allowing an average number of thirty living calves a year by a bull in a pedigree herd, this means that the initial cost of the calf in respect of its sire amounts to £1 plus one-thirtieth of the cost of the maintenance, etc., of the bull. And this is only in respect of the average pedigree herd.

In the first-class herds this cost is considerably higher, though it would seem probable that in the leading herds bulls are kept to a greater age.

An increase in the productive life of pedigree bulls would effect an immediate cash saving to the breeder. If little is known with certainty concerning the reasons for the high annual replacement percentage of cows, still less is known concerning the still higher percentage of bulls. One of the reasons, and perhaps the principal one, is that a bull with increase of age becomes too heavy for the cows to which he is mated, especially since one-third of those cows are yearling heifers, as the results of Part I of this study have shown them to be. Incidentally it might be pointed out that a lowering of the annual replacement percentage of the cows would also enable bulls to be kept to a greater age, and another economy could thereby be effected.

A second reason for the early disposal of so many bulls is that they are not kept according to the rules of good husbandry. Frequently bulls are overfed, and this contributes to undue weight more than does age; bulls also are often kept in small dark boxes without proper exercise, and this leads to bad temper. Both these points do not require further research in order to effect improvement. They can to a great extent be overcome by education.

A third reason is that old bulls frequently become sterile. This may be a result of bad husbandry or it may be genetic. The writers suspect that the genetic aspect of sterility in bulls is of no inconsiderable importance.

A fourth reason is the fact that, by the time a bull has been in use for 2 years, his daughters are coming on for service, and, quite rightly, in many herds there is a natural reluctance to mate him to his own daughters. This would explain why breeders have to obtain a new bull every 2 years. But must they always send the old bull to the butcher and replace it with a young one? At the present time there certainly exists an objection amongst the majority of breeders to the purchase of an old bull. This is probably not altogether without some just foundation, for many old bulls are either too heavy or relatively infertile. Some light on this point would be of value.

A fifth reason—and there are others—may be due to the fact that since "the sire is half the herd" more discrimination is exercised in his selection, and pedigree breeders are continually on the search for something better. This may account for the low average age of the beef bulls, but it certainly does not for the dairy ones. Judged by the progeny

test the breeding value of a bull can, to a certain extent, be assessed by the time he has been in use for 2 years, that is, when he is 3 years of age, for by that time his first calves will be a year old and their quality and rate of maturity can be roughly appraised. But if bulls in beef herds are discarded because they do not transmit desirable qualities to their calves, then it is a great reflection upon the methods of our pedigree breeders that, even after rigid selection by their standards (after all not more than one pedigree bull in ten born is used in a pedigree herd), more than half of the bulls have to be sent to the butcher directly the value of their calves can be assessed. Indeed, it is to be doubted whether any sensible proportion of those bulls which are sent to the butcher at 3 years of age or under go because they have proved themselves begetters of indifferent stock.

The value of a dairy bull cannot be estimated by the progeny test till his daughters have been in milk for at least 6 months, and even then it is better that they should complete a lactation before attempting to evaluate their sire. But granting that some sort of assessment of the sire can be made after his daughters have been in milk 6 months, and granting that the average age at which his daughters first calve is at $2\frac{1}{2}$ years, then we find that something over 80 per cent. of the bulls used in the pedigree dairy herds of this country are disposed of before their value can be known by the surest method available to the constructive breeder—the progeny test.

In the writers' opinion there is room for enquiry into the reasons why so few old bulls are used in the pure bred herds of cattle of this country. But a great deal can be accomplished without any further enquiry, or research based on such enquiry. That the value of the progeny test should not be sufficiently recognised by the commercial breeders of this country is not surprising, since they are concerned with ephemeral things, but that the value of this test should be so wholly unappreciated by the majority of pedigree breeders—the men in whose hands constructive improvement in the quality of our livestock is absolutely dependent—is nothing short of a disgrace to a country with such an honourable record of stock improvement as ours.

Summary.

From the herd book records of six leading breeds of cattle in Great Britain (Shorthorn, Aberdeen-Angus, Hereford, Ayrshire, Jersey and British Friesian) the ages of the parents of 2618 pedigree calves born in 1925 were tabulated.

The average age of cows at calving is found to be 5·722 years ± 0·04, with a standard deviation of 2·816. In four dairy breeds the average age is 5·485 years ± 0·05, while for the three beef breeds it is 6·022 ± 0·05.

The "annual replacement percentage" is the number of heifers drafted annually into a herd or breed as first calvers expressed as a percentage of the total number of actively producing cows in the herd or breed. It is in effect the number required annually to make good the losses, and can be calculated from the average age of the cows at calving. This is found to be at least 27 per cent. and agrees substantially with the work of other investigators.

The need for enquiry into the reasons for such a high replacement figure is stressed. It is pointed out that the ideal annual replacement figure is 10 per cent., but if the existing figure could be reduced by improved methods to only 16·7 per cent. it would result in a saving of £35 per annum in a herd of twenty cows.

Attention is also drawn to the fact that, at the present replacement figure, there is little room for the selection on genetic grounds of females drafted into the herd. A lowering of the replacement percentage would, by giving the breeder a better chance to select his female stock, effect an improvement in the quality of pure breds.

The second part of the paper deals with the average age of bulls at the time their progeny are born. For all the breeds studied this is found to be 3·846 years ± 0·03, with a standard deviation of 1·943. For the four dairy breeds it is 3·687 ± 0·03, and for the three beef breeds it is 4·052 ± 0·04.

The annual replacement figure for all breeds was found to be 54 per cent. For the dairy breeds it is 60 per cent. and for the beef breeds 49 per cent.

The reasons for this are discussed, and emphasis is laid on the fact that the early age at which bulls are disposed of does not permit breeders to employ the progeny test, which is the surest method of stock improvement. In the dairy breeds over 80 per cent. of the bulls are disposed of before their genetic value can be recognised.

No significant difference was found in the proportion of the sexes of the calves in relation to the ages of their parents.

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STUDIES IN TROPICAL SOILS.

I. IDENTIFICATION AND APPROXIMATE ESTIMATION OF SESQUIOXIDE COMPONENTS BY ADSORPTION OF ALIZARIN.

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I. INTRODUCTORY.

STUDIES of soil-genesis by the profile method frequently involve complete chemical analysis of representative horizon samples by familiar acid digestion or fusion methods. Unfortunately, such methods fail to distinguish between components that are combined and those that are uncombined (silica, alumina, ferric oxide and oxides of alkaline earths, and alkali metals). Certain digestion methods perhaps furnish means of determining uncombined *silica* (quartz), but none yields direct estimation of the free sesquioxide components.

In order to gain some insight into the manner in which the different oxide entities (including water) are combined in a rock, clay or soil, it is customary to assume the presence of certain standard primary minerals of constant composition, or of definite secondary products (such as kaolinite, gibbsite, calcite and iron ores), and then to calculate the proportionate amounts of these compounds from the analytical data. In most instances this basal assumption is probably incorrect, particularly for soils and clays that may contain indefinite compounds, for example, hydrous alumino-silicates and hydrous ferro-silicates, for which standard formulae are not available.

An urgent need thus arises for methods whose application directly and definitely leads to the detection and estimation of secondary mineral products in weathered rock material. Failing such methods, studies of soil genesis become largely speculative or superficially descriptive in aspect; they lack the precision necessary for purposes of exact comparison. It is frequently maintained, for instance, and without very definite proof, that the characteristic product of the so-called lateritisation process of rock-weathering under humid tropical conditions is alumina, together with other less characteristic products, such as ferric oxide, manganese oxide and titania, in more or less hydrated state.

Silica is said to occur among the products of extreme lateritisation entirely in the form of secondary quartz, although it is believed to occur in intermediate and less completely weathered material, partly as alumino-silicates, such as kaolin.

Since the commoner chemical agents of digestion fail to differentiate between alumina, ferric oxide and silica in the free state and in dual combination, recourse must be made to other agents that act differently. Among these, dyestuffs have frequently been selected for application under various arbitrary conditions. Unfortunately the complexity of the reactions involved in dye absorption processes, and the difficulty of controlling the various directing influences, have rendered dyestuff methods unattractive to most soil investigators. The historical aspect of the subject has been discussed in this *Journal* by Croucher[1], and need not here be reconsidered.

II. Alizarin adsorption by alumina.

A discovery announced by Schmelev in 1928 has suggested a new dyestuff technique which appears to be particularly suited to the identification and approximate estimation of free alumina in clays and soils. It has been described as follows[2]:

The material is treated in a test-tube with a hot alcoholic solution of alizarin red previously saturated with boric acid at boiling-point, to prevent coloration of alkalies and alkaline earths. The contents of the tube are boiled and decanted, and the residue washed with hot boric-alcohol. The extent to which the residue is stained red indicates the amount of free alumina present.

Gibbsite (hydrargillite) and diaspore do not react[1] with alizarin red, *but they become strongly coloured after heating for 2–5 minutes* in the Bunsen flame. *No liberation of alumina from kaolinite was detected by this method between the temperature limits of 550° and 1100° C.*

The writer has verified these facts, and has elaborated the following tested procedure for application to rock-weathering products, including soils. A modification of the Schmelev technique is rendered necessary,

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[1] Weiser[3, b] has demonstrated that pure alumina, prepared from aluminium amalgam and water, at first shows a large capacity for alizarin adsorption, but that, on keeping for a few days, the hydrous substance "ages," and loses its ability to adsorb alizarin. Impure preparations, containing adsorbed chloride ion, however, were found to age much less rapidly. These observations have obvious bearing on the changes that occur in hydrous alumina liberated during the alteration of minerals under humid climatic conditions, and suggest that the possibility of hydrous alumina remaining active under natural conditions of formation is indeed very remote, and would occur only when anionic concentration was maintained at a high value.

however, by the fact that iron oxide adsorbs alizarin, but only in the fresh, unignited state (see below).

Procedure.

(*a*) *First stage.*

1. Two portions are taken, each of mass 1 gm., of the air-dried material that has been finely ground so as to pass a 100-mesh to the linear inch sieve. One portion is heated to dull redness (800° C.) for 6 minutes in a silica crucible covered by a lid. The other is not heated.

2. Each portion is introduced into a Pyrex glass test-tube (20 cm. long by 3 cm. diameter) containing 20 c.c. of a 0·5 per cent. solution of sodium alizarin sulphonate (alizarin-S) in 80 per cent. alcohol, saturated with boric acid (*p*H 3·2). Adhering particles are washed down the sides of the tubes with 10 c.c. of 80 per cent. boric alcohol. The tubes are fitted with simple condensers, and then heated side by side in a gently boiling water bath for 10 minutes.

3. After settling for 5 minutes, the supernatant liquid in each tube is decanted into a 30 c.c. silica Gooch crucible containing a pad of filter-paper pulp, and fitted into a 1 litre vacuum filtering flask. The solid substance is treated with about 25 c.c. of boric alcohol, boiled, and the whole suspension poured into the crucible, and filtered by suction.

4. Excess of dyestuff is washed out of the sediment with a little boiling boric alcohol, followed by two to ten 25 c.c. portions of boiling distilled water successively added until the runnings come through colourless.

(*b*) *Second stage.*

5. In order now to abstract the adsorbed dyestuff from the stained material, for subsequent colorimetric estimation, a molar aqueous solution of sodium oxalate containing sufficient free oxalic acid to impart a *p*H value of *p*H 3·8 is used. About 30 c.c. of the oxalate solution is poured into each of the tubes, and the pads of paper-pulp, each with its adhering stained solid material, is removed with the point of a rustless steel pen knife and dropped into the solution in the tube. Particles remaining in the Gooch crucibles are washed down through the pores. The contents (50 c.c.) of the tubes in each case are next heated to boiling and settled for 5 minutes.

6. The supernatant liquid (coloured yellow by abstracted alizarin) is poured into a Büchner funnel filter (diameter 6 cm.) and filtered by suction into a clean 1 litre filter-flask. The solid is treated with more

oxalate solution, the mixture boiled, poured on to the filter, and washed with two or three 25 c.c. portions of boiling oxalate solution. The washings (100 to 130 c.c.) are finally transferred to a graduated cylinder, and made up to a definite volume (140 c.c.) with rinsings of distilled water used for washing out the flask.

7. The concentration of dyestuff in the final solutions is measured against a standard in a Duboscq colorimeter. The results furnish estimates both of the alumina content and of the iron oxide content of the material.

Notes.

(a) *First stage.*

1. It is imperative that the crucible in which the material is ignited be covered by a lid, otherwise serious loss due to spurting may occur. Moistening the powder with pure alcohol aids in preventing loss.

2. Aqueous alcohol is preferred to water as dye solvent, because it lessens filtration difficulties with the more sticky clays. Sodium alizarin sulphonate[1] is preferred to alizarin because it is much more soluble in water. It is nearly insoluble in pure alcohol, however, hence 80 per cent. aqueous alcohol is advocated. Boric acid serves to buffer the dye solution against changes in reaction induced by admixture with soils or with alkali-generating minerals. Furthermore, an acidic reaction in the soil-dyestuff system favours adsorption of the alizarin-sulphonate radicle. Gentle ebullition serves to agitate the mixture of solid and dye solution.

3. Silica Gooch crucibles are recommended because their pores are large, and filtration through them is rapid. Paper-pulp does not adsorb the dyestuff. In order further to aid rapid filtration, especially in the case of clays, the addition of a little pure dehydrated silicic acid or powdered hard glass, together with a little kieselguhr or Filter-cel, is advised. These materials should be added to the contents of the tubes

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[1] Weiser's investigations on alizarin-alumina lake formation (3, *a* and *b*) have shown that the lake contains adsorbed alizarate ion, and that the degree of adsorption is diminished in the presence of acid ions (sulphate, oxalate), especially at acidic reactions, and increased by metal ions (calcium), especially at alkaline reactions. His quantitative results demonstrate that 1 gm. of alumina, occurring in a freshly prepared hydrous alumina sol, at pH 3·3, adsorbs 0·318 gm. of alizarin-S. Calculations based on our own results show that our most reactive solid hydrous alumina preparations adsorb, per gram of alumina, about 0·063 gm. of alizarin-S, which is equivalent to 12·6 c.c. of a 0·5 per cent. solution of the dyestuff. Hence the amount of dyestuff solution (20 c.c.) employed in each of our determinations of the alumina content of clays and soils (1 gm.) would appear to be quite adequate.

during the second boiling with alcohol. None of them appreciably adsorbs the dyestuff.

4. Boiling distilled water effectively removes the last traces of un-adsorbed dye. After thorough water-washing, pure specimens of white alumina, under the prescribed conditions, acquire a bright scarlet colour.

(b) Second stage.

5. Sodium oxalate-oxalic acid solution appears to be a most satisfactory dye-abstracting agent, by virtue of its ability to dissolve the surface layer of alumina or ferric oxide at which the dyestuff is combined. At pH 3·8 the dye exhibits a pure yellow colour. If the final oxalate extract is not coloured full yellow, a little solid oxalic acid should be added. (Alizarin solutions become pinkish at reactions more alkaline than pH 4·5.)

6. The use of oxalate solution throughout the whole of the dye-abstracting operations is necessary in order to attain rapid filtration. Water alone should on no account be used, even in the later washings, since it peptises clay, which causes the filter to clog and the filtrate to become cloudy. In many cases the final yellow filtrate is slightly turbid, chiefly owing to the formation of calcium oxalate. It is then necessary to refilter after adding a little kieselguhr or Filter-cel before attempting colorimetric comparison.

7. A satisfactory standard for comparison in the colorimeter is made by mixing 1 c.c. of the original 0·5 per cent. dyestuff solution with 100 c.c. of the oxalate extracting solution, and diluting to 140 c.c. with water. The mixture is then diluted 50 c.c. to 100 c.c. with water. This standard dye solution ($\frac{1}{2}$ c.c. dye standard) is very suitable for obtaining colour matches in the middle range of a Duboscq colorimeter furnished with cylindrical cups of depth 5 cm. Most of the soil dye extracts require no dilution in order to obtain good matches with the standard, but in extreme cases (highly aluminous materials) appropriate dilution of the extract is necessary. Attempts to prepare a permanent colour standard were not successful. Most stable yellow-coloured compounds (acidic ferric chloride, bichromates, picric acid, etc.) exhibit tinctorial properties differing from those of alizarin. In examining a number of soils daily, fresh alizarin standards should be made at intervals of 3 or 4 days; tests have shown that the colour value of the standard deteriorates somewhat on keeping.

Iron solubility. A serious complication arises when ferruginous materials are examined by the procedure above described, through the

dissolving of iron oxide by the oxalate extracting solution. The colour thereby imparted to the solution by the ferro-oxalate ion is yellow, but it is greener in tint than alizarin yellow. It is necessary in all cases to correct for this iron solubility of ferruginous materials, by performing a "blank" oxalate extraction on 1 gm. of the material, both fresh and ignited, omitting, of course, the first stage dyestuff treatment. In order to obtain good colour matches with ferro-oxalate solutions a trace of blue dye (royal blue ink is suitable) must be added to the alizarin colour standard.

III. Some preliminary results obtained.

The procedure was first applied to a series of materials other than soils. The following main results were obtained (Table I).

(a) Alumina.

1. Commercial alumina, bauxites, gibbsitic laterites and alumino-siliceous laterites gave appreciable positive results *only after ignition* at red heat. The uptake of alizarin was then very marked.

2. Artificially prepared specimens of hydrous alumina gave positive results *both before and after ignition*, excepting alumina prepared by the slow action of carbonic oxide on solutions of sodium aluminate, which gave definite positive results *only after ignition*. The magnitude of alizarin adsorption by the fresh, unignited artificial substances *varied with the method of preparation*, and was generally considerably larger than that shown by the ignited material.

(b) Ferric oxide.

3. Specimens of artificially prepared ferric oxide gave high positive results for the *fresh, unignited material, but showed little alizarin adsorption after ignition*. The magnitude of alizarin uptake was much greater for fresh, unignited ferric oxide than for ignited alumina, but varied greatly with the method of preparation.

4. Specimens of the more heavily hydrated preparations of ferric oxide ("limnite" type), even *after ignition*, showed appreciably greater alizarin adsorption than those of the less hydrated types (haematite, turgite).

(c) Minerals.

5. The following *minerals* and other materials gave negligible or no positive results, neither before nor after ignition: quartz, potash felspar, soda-lime felspar, white and black micas, hornblende, augite, olivine,

magnetite, apatite, calcite, dolomite, phosphate-rock, gypsum, chalk, kaolinite, titania, corundum, bentonite, fuller's earth, hydrous silica, kieselguhr, Filter-cel, and highly humic organic manure.

Note. In those cases where alkali is generated through the hydrolysis of minerals (*e.g.* felspars, hornblende, olivine, apatite) the colour of the alizarin solution after boiling with the powdered solid was usually deep wine-red, despite the presence of boric acid. Precipitation of the dyestuff on the solid particles may then occur, leading to erroneous results, unless filtration and washing are immediately and rapidly performed.

Iron solubility.

6. Iron oxide preparations exhibited marked solubility in the oxalate extracting solution, necessitating the performance of careful "blank" tests (Table I). The coloration due to iron solubility in oxalate solutions

Table I. *Results of alizarin tests on pure sesquioxides.*

| | Water loss, on heating | | | Alizarin values (units per gm. of material)* | | | | | | | |
| | % air dry soil | | % oven dry soil | Fresh, unignited material | | | | Ignited material | | | |
Material	On oven drying and ignition	On oven drying	On ignition alone	Sesqui-oxide	Oxalate extract	Differ-ence	Cor-rected for water content	Sesqui-oxide	Oxalate extract	Differ-ence	Cor-rected for water content
(a) ALUMINA:											
(1) Preparation 1	62·5	21·8	51·3	547	19	528	1408	339	14	325	868
(2) " 2	34·9	0·0	34·9	717	14	703	1080	607	13	594	913
(3) " 3	47·7	19·7	34·9	483	18	465	889	442	15	427	816
(4) " 4	67·8	27·7	55·5	571	17	554	1720	303	15	288	895
(5) " 5	34·0	1·1	33·3	37	17	20	30	460	13	447	678
(6) Commercial	33·8	0·4	33·5	23	10	13	19	477	12	465	702
(7) Bauxite 3	29·9	0·2	29·8	23	19	4	6	424	10	414	596

Means for gibbsitic types: fresh 18   Means: ignited 660
Means for heavily hydrated types: fresh 1280   Means: ignited 873

(b) FERRIC OXIDE:											
(1) Preparation 1	33·0	24·5	11·2	1401	195	1206	1800	30	17	13	20
(2) " 2	20·5	0·0	20·5	1328	186	1142	1437	42	20	22	27
(3) " 3	35·6	22·1	17·3	1388	205	1183	1336	42	16	26	40
(4) " 4	44·8	24·9	26·5	1621	246	1375	2490	95	54	41	72
(5) Commercial	15·5	10·6	5·5	1182	167	1015	1399	34	10	24	29

Means for lightly hydrated types: fresh 1500   Means: ignited 30
Means for heavily hydrated types: fresh 2500   Means: ignited 70

* Unit = Colour value per hundredth-centimetre depth of a standard containing 1 c.c. of 0·5 per cent. solution of alizarin-S diluted to 140 c.c., as viewed in a Laurent Duboscq colorimeter furnished with a 6 cm. scale.

Preparation 1. Metal chloride + ammonia, room temp.; then boiled in presence of ammonium chloride.
     "   2. .Metal chloride + ammonia, room temp.; then oven dried.
     "   3. Metal chloride + ammonia, boiling temp.; then air dried.
     "   4. Metal chloride + ammonia, ice temp.; then air dried.
     "   5. Sodium aluminate + carbonic oxide, room temp.; then air dried.

Note. According to Morrow Campbell (4), hydrous ferric oxide preparations made by methods 1, 2 and 3 are relatively lightly hydrated, and yield *red* products on ignition, whereas that made by method 4 is more heavily hydrated, and yields a *black* product on ignition.

was much the greatest for the fresh, unignited material. Its intensity increases with the degree of acidity of the extracting solution, which thus imposes a lower limit to the pH value to which the solution may be brought (pH 3·8). *Ignited* ferric oxide specimens showed slight but appreciable variations in iron solubility, depending apparently on some property conferred by the conditions of preparation. Thus, iron oxide specimens that are lightly hydrated and possess dark brown colours yield bright red ignited products exhibiting relatively low solubility in oxalate solutions, whereas paler coloured, heavily hydrated preparations yield black ignited products exhibiting greater solubility in oxalate solutions. In general, the lightly hydrated preparations of ferric oxide appear to be less reactive than the more heavily hydrated preparations.

Table II. *List of specimens examined.*

Number and name	Locality, origin and description
1. Bauxite rock 3	British Guiana, Demerara R., Trewern mine. Derived from dolerite. Buff-cream (6).
2. Pisolitic laterite	British Guiana, N.W. District, Issorora. Derived from epidiorite. Pale red (4).
3. Cuba red soil	Cuba, Oriente Province, Nipe, Guara Estate. Derived from serpentine rock. Contains abundant shot-like concretions. Dark red (1).
4. Bermuda red soil	Bermuda, B.W.I., Paget East, Agric. Station. Derived from shelly calcareous sandstone. Subsoil. Dark red (1).
5. Anguilla red soil	Virgin Islands, B.W.I. Derived from coral rock. Somewhat concretionary. Dark red (1).
6. White bauxitic clay	(See 1.) Occurs under hard bauxite rock. White (10).
7. Indian laterite rock	Madras. Typical vermicular laterite. Red (2).
8. Hawaii red soil	Oahu Is., Koolan Plateau. Derived from basalt and basic volcanic ash. Dark red (1).
9. Java red soil	Batavia, Pasar Minggoe. Derived from trachytic tuff. Contains shot-like concretions. Dark red (1).
10. Dominica red clay	Dominica, B.W.I., Imperial Rd., Mount Joy Estate. Derived from hypersthene andesite. Subsoil. Red (2).
11. Red bauxitic clay	(See 1 and 6.) Overburden of bauxite rock. Pale red (5).
12. Barbados red soil	Barbados, B.W.I., St James, Apes Hill Estate. Derived from coral rock containing intercalated andesitic volcanic ash. Red-orange (3).
13. Jamaica red soil, F	Jamaica, B.W.I., Trelawney, Friendship Estate. Derived from Oceanic limestone. Contains abundant shot-like concretions, which have been mainly removed. Subsoil. Tawny yellow (7).
14. Trinidad yellow soil	Trinidad, B.W.I., Naparimas, Harmony Hall Estate. Derived from old river alluvium. Greenish yellow (8).
15. Jamaica red soil, B	(See 13.) St Elizabeth, Bogue Pen. Derived from Oceanic limestone. Concretions removed. Subsoil. Yellow-orange (4).
16. Pink bauxitic clay	(See 1, 6 and 11.) Underburden of bauxite rock. Pale rose-pink (5).
17. Kaolin	Commerical specimen. Baird and Tatlock, London. White (10).

Table II (*contd*).

Number and name	Locality, origin and description
18. Kaolinitic clay	Commercial "colloidal clay." Townson and Mercer, London. White (10).
19. Trinidad pale red soil	Trinidad, B.W.I., Naparimas, Cedar Hill Estate. Derived from red weathering clay of Middle Miocene age. Brownish buff (6).
20. Dominica yellow soil	Dominica, B.W.I., Imperial Rd., Riverdale Estate. Derived from hypersthene andesite under annual rainfall of 150 inches. Tawny greenish yellow (7).
21. Barbados yellow soil	Barbados, B.W.I., Scotland District, Forster Hall Estate. Derived from sedimentary clay of Cretaceous age. Greenish grey (8).
22. Trinidad white clay	Trinidad, B.W.I., St Augustine, Cotton Research Station. Detrital clay derived from quartzose schist of pre-Cretaceous age. Subsoil. Pale brown-buff (6).
23. London clay	London, England, Oxford Street, Selfridge's. Deep excavation. Subsoil. Yellowish grey (9).
24. Trinidad red earth	Trinidad, B.W.I., Maracas Valley, Northern Range. Derived from quartzose schist of pre-Cretaceous age. Subsoil. Red (2).
25. Bentonite	Specimen obtained from Imperial Institute, London. Greyish white (1).
26. Trinidad pink clay	(See 22.) Overlying layer below humic soil. Pale orange-red (4).
27. Antigua chocolate soil	Antigua, B.W.I., Southern Hills, Bendal's Estate. Derived from valley alluvium of weathered andesitic ash. Dark pinkish grey (9).
28. Antigua grey soil	Antigua, B.W.I., Central Plain, Tomlinson's Estate. Derived from aqueous siliceous tuff of Pleistocene age. Resembles bentonite. Pale pinkish grey (9).
29. Indian pale red soil	Madras, Nellore, Gudur. Derived from quartzose laterite. Pale, dull red-orange (4).
30. Fuller's earth	Commercial specimen. Baird and Tatlock, London. Greenish grey (9).
31. Congo pink clay	Belgian Congo, Bangala, origin unknown. Subsoil. Pale rose-pink (5).

Note. (1) The figure in brackets after the colour description indicates intensity of *red colour*, based on an arbitrary scale of 1 to 10, and estimated by inspection of the moistened material.

(2) None of the soils contains more than 2 per cent. of organic matter. More than half of them (subsoils) contain negligible amounts, and only one-third of them above 1 per cent. of organic matter.

IV. Application to clays and soils.

The procedure was next applied to a series of clays and soils forming part of an accumulated collection of specimens, procured mainly in the British West Indies. The specimens examined are briefly described in Table II. Data referring to their chemical composition and their alizarin values are present in Tables III and IV, the first of which contains results obtained with the *whole* material, ground to pass a 100-mesh sieve, and

Table III. *Results of alizarin tests on whole soils.*

Materials (Arranged in order of total sesquioxide/silica ratio)	SiO₂	Al₂O₃	Fe₂O₃	H₂O	$\frac{SiO_2}{R_2O_3}$	$\frac{H_2O}{SiO_2}$	Material Fresh	Material Ignited	Oxalate extract Fresh	Oxalate extract Ignited	Corrected values Fresh	Corrected values Ignited	Free Al₂O₃ (×0·150)	Free Fe₂O₃ (×0·066 or ×0·040)	Sum Free R₂O₃	$\frac{Free\ R_2O_3}{SiO_2}$
1. Bauxite rock 3	1·4	62·1	2·7	29·8	46·3	21·3	23	424	19	10	4	414	62·1	0·3	62·4	44·57
2. Pisolitic laterite	2·0	58·2	9·6	26·7	33·9	13·3	52	323	14	9	38	314	47·1	2·5	49·6	12·40
3. Cuba red soil	7·7	16·7	62·3	12·9	10·3	1·67	163	67	12	17	151	50	7·5	8·3	15·8	2·05
4. Bermuda red soil	27·2	35·8	16·7	20·8	1·93	0·76	168	89	40	14	128	75	11·2	5·1	16·3	0·60
5. Anguilla red soil	30·6	27·4	27·9	14·4	1·81	0·47	114	48	31	18	83	30	4·5	3·3	7·8	0·22
6. White bauxitic clay	26·4	46·3	0·8	22·8	1·56	0·86	10	215	2	5	8	210	31·5	0·5	32·0	1·21
7. Indian laterite rock	34·0	21·7	31·5	11·9	1·53	0·35	116	90	10	6	106	84	12·6	7·1	19·7	0·58
8. Hawaii red soil	30·0	25·8	20·1	17·1	1·27	0·57	139	39	25	19	114	20	3·0	4·6	7·6	0·25
9. Java red soil	37·5	37·5	10·2	15·0	1·21	0·40	205	27	14	8	91	19	2·8	7·6	10·4	0·21
10. Dominica red clay	37·9	27·7	18·0	13·7	1·21	0·36	135	35	22	7	113	28	4·2	7·6	11·8	0·31
11. Red bauxitic clay	37·4	41·3	2·7	15·0	1·17	0·43	19	81	3	5	16	76	11·4	1·1	12·5	0·33
12. Barbados red soil	38·8	27·8	17·3	13·2	1·16	0·34	169	34	14	10	155	24	3·6	6·2	9·8	0·23
13. Jamaica red soil, F	37·9	31·8	11·2	17·3	1·08	0·46	122	38	18	13	104	25	3·7	5·7	9·4	0·25
14. Trinidad yellow soil	41·4	32·0	12·4	12·5	1·07	0·30	56	18	30	3	26	15	2·2	1·7	3·9	0·10
15. Jamaica red soil, B	42·0	29·2	12·2	15·9	0·99	0·38	131	28	12	18	119	10	1·5	4·8	6·3	0·15
16. Pink bauxitic clay	42·3	38·0	1·8	14·4	0·94	0·34	16	36	3	4	13	32	4·8	0·8	5·6	0·13
17. Kaolin	46·7	38·3	3·5	10·0	0·89	0·21	17	5	2	1	15	3	0·4	0·6	1·0	0·02
18. Kaolinitic clay	47·3	37·2	4·0	11·5	0·87	0·24	24	4	2	2	22	3	0·4	0·9	1·3	0·03
19. Trinidad pale red soil	49·5	30·3	10·2	9·5	0·82	0·19	92	25	31	9	61	16	2·4	2·4	4·8	0·10
20. Dominica yellow soil	47·2	16·9	18·2	16·2	0·74	0·34	105	39	30	16	75	23	3·5	3·0	6·5	0·14
21. Barbados yellow soil	59·4	22·7	12·0	5·8	0·58	0·09	40	15	21	8	19	7	1·0	0·8	1·8	0·03
22. Trinidad white clay	60·4	29·6	5·0	4·6	0·57	0·07	30	11	6	4	24	7	1·0	1·0	2·0	0·03
23. London clay	59·1	22·8	11·3	5·1	0·57	0·08	57	15	15	10	42	5	0·7	1·7	2·4	0·04
24. Trinidad red earth	58·9	18·6	14·0	7·0	0·55	0·11	65	29	33	17	32	12	1·8	2·1	3·9	0·07
25. Bentonite	57·2	21·3	8·9	8·6	0·53	0·15	18	7	4	3	14	4	0·6	0·6	1·2	0·02
26. Trinidad pink clay	63·3	21·9	8·1	5·2	0·48	0·08	53	18	18	13	35	5	0·7	1·4	2·1	0·03
27. Antigua chocolate soil	62·7	18·0	12·2	5·7	0·48	0·09	42	19	17	4	25	15	2·2	1·7	3·9	0·06
28. Antigua grey soil	64·4	17·2	8·6	8·6	0·40	0·13	19	8	9	4	10	4	0·6	1·6	2·2	0·01
29. Indian pale red soil	66·8	12·7	12·9	6·6	0·39	0·10	57	20	16	5	41	15	2·2	1·6	3·8	0·06
30. Fuller's earth	68·2	14·2	5·9	7·8	0·29	0·11	27	6	8	4	19	2	0·3	0·8	1·1	0·02
31. Congo pink clay	77·1	11·8	6·2	4·4	0·23	0·05	17	6	6	3	11	3	0·4	0·7	1·1	0·01

Table IV. Results of alizarin tests on clay fractions.

Clay fraction (Arranged in order of total sesquioxide/silica ratios)	Chemical composition SiO₂	Al₂O₃	Fe₂O₃	H₂O	Ratios SiO₂/R₂O₃	SiO₂/H₂O	Alizarin values — Clay Fresh	Clay Ignited	Oxalate extract Fresh	Oxalate Ignited	Corrected values Fresh	Corrected Ignited	Free Al₂O₃ (×0.150)	Free Fe₂O₃ (×0.066 or ×0.040)	Sum Free R₂O₃	Ratio Free R₂O₃/SiO₂	(Arranged in order of free sesquioxide/silica ratios)
1. Bauxite rock 3	8.0	11.3	67.9	12.0	No clay fractions separated												—
2. Pisolitic laterite	24.9	47.1	7.6	20.8													—
7. Indian laterite rock	27.8	33.8	17.5	18.6													—
3. Cuba red soil	32.0	33.0	16.0	13.1	9.90	1.50	173	76	13	11	160	65	9.7	8.6	18.3	2.29	Cuba red soil
6. White bauxitic clay	29.9	28.2	23.2	12.3	2.19	0.83	52	227	4	7	48	220	33.0	3.2	36.2	1.45	White bauxitic clay
4. Bermuda red soil	33.8	17.6	20.4	12.8	1.84	0.67	284	74	52	20	232	54	8.1	9.3	17.4	0.63	5. Bermuda red soil
5. Anguilla red soil	32.0	28.3	8.3	10.2	1.53	0.41	126	24	31	9	95	15	2.2	3.8	6.0	0.19	Dominica red clay
10. Dominica red clay	29.9	28.9	16.6	14.7	1.53	0.41	153	22	12	14	141	8	1.2	9.4	10.6	0.35	Java red soil
8. Hawaii red soil	34.3	28.9	18.7	13.6	1.50	0.37	147	37	22	12	125	25	3.7	5.0	8.7	0.25	Hawaii red soil
24. Trinidad red earth	36.2	40.0	20.9	13.9	1.36	0.28	117	18	45	6	72	12	1.8	4.8	6.6	0.18	10. Jamaica red soil, F
11. Red bauxitic clay	38.2	29.7	8.5	12.3	1.26	0.38	49	55	4	8	45	37	5.5	3.0	8.5	0.24	Pink bauxitic clay
13. Jamaica red soil, F	38.6	27.2	10.7	14.7	1.19	0.35	130	38	30	13	100	25	3.7	5.5	9.2	0.24	Dominica yellow soil
9. Java red soil	38.3	25.9	16.2	12.6	1.19	0.36	170	29	17	19	153	10	1.5	8.4	9.9	0.26	Red bauxitic clay
15. Jamaica red soil, B	41.8	34.8	27.6	11.6	1.12	0.29	138	36	5	5	128	45	6.7	2.4	9.1	0.23	Jamaica red soil, B
16. Pink bauxitic clay	40.2	29.7	3.0	7.6	1.07	0.34	41	50	10	13	89	23	3.4	5.1	8.5	0.20	15. Anguilla red soil
12. Barbados red soil	40.0	27.6	9.0	13.1	1.01	0.37	107	19	18	9	101	10	1.5	3.6	5.1	0.13	Trinidad red earth
29. Indian pale red soil	44.7	39.3	11.0	15.4	0.98	0.28	135	15	34	4	11	11	1.6	4.0	5.6	0.13	Barbados red soil
17. Kaolin	45.7	32.6	3.4	9.3	0.92	0.25	17	5	6	5	65	0	0.0	0.4	0.4	0.01	Indian pale red soil
26. Trinidad pink clay	48.6	28.5	13.4	6.7	0.85	0.15	82	34	17	12	162	5	0.7	0.4	3.3	0.07	Antigua chocolate soil
20. Dominica yellow soil	46.9	35.1	5.1	5.1	0.84	0.28	180	17	18	8	11	26	3.9	2.6	10.4	0.22	20. Trinidad pink clay
18. Kaolinitic clay	46.7	21.0	11.3	12.4	0.82	0.33	20	5	6	5	56	11	1.6	6.5	0.6	0.01	Trinidad pale red soil
19. Trinidad pale red soil	51.9	28.5	9.1	6.3	0.66	0.18	86	9	30	6	35	1	0.1	0.6	3.8	0.07	Fuller's earth
22. Trinidad white clay	54.8	22.8	10.1	6.8	0.61	0.12	44	17	9	8	51	9	1.3	2.2	1.5	0.03	London clay
23. London clay	59.1	21.0	9.8	6.2	0.57	0.08	61	5	10	8	0	11	1.6	1.4	3.3	0.05	Trinidad yellow soil
25. Bentonite	57.2	17.4	21.8	7.9	0.52	0.21	8	20	8	9	28	5	0.1	2.0	0.1	0.01	25. Barbados yellow soil
14. Trinidad yellow soil	62.7	16.9	10.7	6.8	0.44	0.10	42	11	14	6	57	11	1.6	1.9	3.5	0.05	Trinidad white clay
27. Antigua chocolate soil	60.3	5.1	8.5	4.4	0.44	0.11	72	26	15	5	12	5	0.7	3.8	4.5	0.07	Kaolin
21. Barbados yellow soil	61.8	21.8	6.0		0.43	0.10	25	12	13	9	57	17	2.5	0.5	3.0	0.05	Kaolinitic clay
30. Fuller's earth	58.8	14.8			0.43	0.13	51	4	6	5	12	—	1.0	1.7	2.7	0.05	Congo pink clay
28. Antigua grey soil	63.9	15.7			0.38	0.11	13	6	6	4	—	0	0.3	0.3	0.3	0.01	30. Bentonite
31. Congo pink clay	77.1	11.1			0.22	0.06	18	6	6	6	12	0	0.0	0.8	0.8	0.01	Antigua grey soil

the second, results for *clay fractions*, separated (after preliminary sifting through a 1 mm. sieve) by violent mechanical stirring followed by conventional sedimentation from water containing sodium carbonate as peptising agent.

In the tables, the clays and soils are arranged in order of decreasing sesquioxide/silica ratios. (It will be noticed that these approximately follow the combined water/silica ratios.) Alizarin values, both for the materials and their oxalate extracts, are fully recorded.

From the corrected alizarin values, apparent free alumina and free iron oxide contents have been calculated on the following assumptions.

1. The *free alumina* component of the products of rock decomposition and weathering occurs mainly as the crystalline tri-hydrate (gibbsite or hydrargillite). Direct microscopical evidence in support of this contention has been obtained by several investigators (notably Bauer, Lacroix, J. B. Harrison and Morrow Campbell). It is substantiated by the fact that alumina liberated by the action of carbonic oxide on alkali aluminate solutions is of the gibbsite type, being spontaneously separated by a process which closely resembles the natural changes occurring during the alteration of certain aluminous minerals, and during the circulation of ground water containing soluble rock residues. Adsorption of alizarin by fresh, unignited clays and soils probably, therefore, cannot be ascribed to free alumina, since fresh gibbsite does not adsorb alizarin.

2[1]. Abundant evidence of the occurrence of *hydrous (colloidal) iron oxides* in clays and soils, however, has been accumulated. The characteristic brown, red and yellow colours of earthy materials are generally believed to be due to their presence. Because fresh hydrous iron oxide readily adsorbs alizarin, the alizarin value of an unignited clay or soil may be regarded solely as an approximate measure of its content of free iron oxide, although the degree and state of hydration of this component may partly decide the magnitude of the alizarin uptake.

3. On the other hand, the alizarin value of an *ignited* clay or soil may be taken solely as an approximate measure of its free (gibbsitic) alumina content, because alizarin is adsorbed by ignited gibbsite but not appreciably by ignited iron oxide.

[1] It appears probable that pure natural anhydrous *haematite* takes up negligible quantities of alizarin-S. If such "inactive" iron oxide occurs commonly in clays and soils, then the contents of iron oxide calculated from alizarin values given in Tables III and IV would appear in many instances to be too low.

Calculating factors.

(*a*) The factor (0·15) used for calculating approximate free alumina contents from alizarin (ignited) values is derived from the quantitative results obtained in the preliminary examination of natural and artificial preparations of alumina (Table I), in which it is seen that the truly gibbsitic types (specimens 5, 6 and 7) adsorb, on an average, 660 standard dye units per gm. of hydrous alumina, or one dye unit per 0·150 gm. of alumina.

(*b*) The factors (0·066 and 0·040) used for calculating approximate free iron oxide contents are based on the preliminary observations that (i) specimens of the lightly hydrated type (Nos. 1, 2, 3, 5), which yield a *red* product on ignition, adsorb, on an average, 1500 dye units per gm. of anhydrous ferric oxide, or one dye unit per 0·066 gm. of ferric oxide, and (ii) specimens of the more heavily hydrated type (No. 4), which yield a *black* product on ignition, adsorb about 2500 dye units per gm., or one dye unit per 0·040 gm. of anhydrous ferric oxide[1]. (In order to differentiate between the two main types of iron oxide that may occur in the clays and soils examined, a note was consequently made of the colour of the ignited product in each case.)

The approximate percentage amounts of free alumina and free iron oxide are added together, and the ratios of the total *free sesquioxide* to free silica are calculated (last column). Finally, the specimens are re-arranged in order of decreasing free sesquioxide/silica ratios.

In practice, it was found expedient to examine six soils in a batch, requiring one day's work for complete estimation of approximate contents of free alumina and free iron oxide. This allowed colorimetric comparisons to be made in the afternoon of the same day, provided adequate amounts of the requisite solutions of dyestuff, boric alcohol and oxalate had previously been prepared. The mechanical operations may be satisfactorily performed by a laboratory assistant possessing little previous training.

[1] (*a*) The factor used for calculating *alumina* contents appears to be satisfactory, so far as our present limited experience goes. Its employment in the analysis of synthetic mixtures of hydrous oxides examined in order deliberately to test the quantitative precision attainable by the procedure described, usually gave the desired results. On the other hand (*b*) the factors used for calculating *ferric oxide* contents must be regarded at present merely as tentative and subject to further correction in the light of future studies of specimens of characteristic iron oxide minerals now being procured. The values of the iron oxide factors were derived solely from determinations made on artificially prepared specimens; they are not altogether satisfactory, even when applied to the analysis of some synthetic mixtures containing different known amounts of these specimens.

V. Conclusions based on the experimental results.

1. Perhaps the most striking feature indicated by the results obtained for the whole clays and soils (Table III) is the surprisingly small magnitudes of their free alumina contents. Bauxite, laterite and white bauxitic clay alone appear to contain more than 12 per cent. of free alumina. Among the soils, the Bermuda red soil contains the greatest amount of free alumina (11·2 per cent.). Most of the other tropical red soils apparently contain less than 5 per cent. of free alumina. One of the yellow soils (from the rainy uplands of Dominica) contains as much free alumina as most of the red soils.

2. Similarly, most of the soils seem to possess only a medium proportion of their total iron oxide content in the form of free hydrous oxide. Even in the highly ferruginous Cuba soil, less than one-eighth of the total iron oxide appears to be uncombined. The highest proportional amount of free iron oxide occurs in the bauxite and laterite samples, although certain of the tropical red soils that yield bright red ignited products (Java, Dominica, Jamaica F) contain a relatively high content of free iron oxide as compared with that which is combined.

3. The final sequence based on decreasing *free sesquioxide*/silica ratios brings together the lateritic materials and the tropical red soils into one group, followed by the less geologically typical tropical soils, including pale red and yellow soils. The more characteristic kaolinitic materials fall naturally into a third group. This sequence thus appears to be more satisfactory than that based on *total sesquioxide*/silica ratios.

4. The results obtained for *clay fractions* (Table IV) indicate that their free alumina contents are even less in amount than those of the whole soils. This observation would seem to be in accordance with mineralogical evidence, which demonstrates that gibbsite tends to segregate into pisolitic concretions of macroscopic magnitude, and might therefore occur mainly in the coarser fractions.

5. The free *iron oxide* contents of the clay fractions, on the other hand, appear to be relatively higher than those of the whole material in the majority of cases. In some examples of red soils where this difference is not very pronounced (Cuba, Anguilla, Jamaica) the whole soils are characterised by the presence of shot-like concretions relatively rich in iron oxide.

6. The sequences based on the ratios of sesquioxide to silica for the *clay fractions* often differ appreciably from those based on the ratios for the whole soils. Thus the three Trinidad detrital materials (red earth,

white and pink clays) exhibit *greater* sesquioxide to silica ratios for the clay fractions, which implies that the whole materials contain considerable amounts of siliceous sand, a conclusion supported by the fact that they have been derived from a highly quartzose schist. The same conclusion applies also to the Indian pale red soil, and, in lesser degree, to the Dominica red soil.

On the other hand, the Bermuda, Anguilla, Java and Barbados red soils show slightly *less* sesquioxide to silica ratios in the clay fractions, which implies that the whole soils contain sand fractions relatively rich in sesquioxides rather than silica. This conclusion applies also to the Trinidad and the Barbados yellow soils, and even to the kaolinitic clay. Hence the separate examination of clay fractions, as well as of the whole materials, may furnish evidence of their origin and relationships, although just as reliable information alternatively might have been deduced from determinations of free and combined *silica* in the whole materials.

VI. Suggestions for further research.

1. The alizarin-adsorption method herein described and applied in a preliminary examination of various rock, clay and soil materials, appears to provide a suitable means of investigating the genesis of soils, and is being applied in profile studies. It is hoped thereby to alleviate the tedium of detailed quantitative chemical analysis.

2. The possible significance of the relatively high free iron oxide content of certain tropical soils in relation to acidity and ionic exchange processes (particularly those involving phosphate ion) merits further study; this should be greatly facilitated by the application of the alizarin method to the identification and estimation of the iron oxide component.

3. A study of the effects of strongly heating soils and clays that have been found to contain appreciable free iron oxide and free alumina, on their capacity for ionic exchange (particularly phosphate ion), might reveal interesting comparisons with results obtained with the unignited materials.

4. The application of results obtained by a detailed investigation of the sesquioxide components of clays and soils to a study of their physical properties might be greatly expedited by the application of the alizarin procedure.

5. Alizarin may not be the best or only reagent for use in attempts to identify and to measure the sesquioxide component of clays and soils. Preliminary trials have indeed demonstrated that haematoxylin may be

even more serviceable, and this possibility is being further examined. Other suitable dyestuffs may also be tested, including some that are believed to react specifically with siliceous components.

6. Materials containing appreciable amounts of sesquioxides furthermore have been found to adsorb easily recognisable ions other than dyestuff ions. Among these, bichromate ion and arsenate ion particularly deserve attention; their estimation is not difficult, and the adsorption process may easily be controlled. Preliminary trials have shown that they are applicable to soil study.

7. Whilst the suggested methods for investigating the sesquioxide component of clays and soils may not furnish quantitative results of the highest degree of accuracy, they may nevertheless be employed in a rapid preliminary examination of large numbers of specimens, from which representative materials may the more easily be selected. These selected specimens may then be submitted to more precise, specific chemical methods of quantitative analysis, particularly those which determine the sesquioxide content of acidic extracts obtained under controlled conditions.

8. The alizarin method may be applied to a study of the formation and properties of artificial zeolites, prepared from sols of alumina and of silica under different controlled conditions, followed by different specific treatments. The state of occurrence of the alumina component in the various resulting products might easily be determined by application of the alizarin procedure. Work along these lines has already been started.

VII. SUMMARY.

1. An alizarin-adsorption method discovered by Schmelev has been successfully applied, with suitable modification, to the identification and approximate estimation of the free sesquioxide components of clays and soils.

2. Details of the tested procedure are presented, together with some preliminary results obtained.

3. The free alumina component of clays and soils appears to adsorb alizarin only after ignition, whereas the free iron oxide component adsorbs it only in the fresh, unignited state. Hence, by applying the procedure to the fresh, as well as to the ignited material, an approximate estimate of both components may be accomplished in a relatively short time.

4. The possibilities of applying the procedure in studies of soil genesis,

ionic exchange processes in soils, and some soil physical properties, are briefly stated.

5. An outline of various similar procedures, involving the use of other reagents, is presented.

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A NEW VIEW OF THE EFFECT OF TEMPERATURE ON MILK PRODUCTION IN DAIRY COWS.

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(With Seven Text-figures.)

INTRODUCTION.

THERE is still much difference of opinion as to the effect of temperature on milk production from the point of view of its effect on both quantity and quality at successive milkings or on successive days.

Bender[1], working in co-operation with the Walker-Gordon Laboratories, found that humidity, not temperature, appeared to affect milk production and, continuing, remarked that high-producing animals were more susceptible to humidity variations than were low-producing ones.

Speir[2], in the concluding remarks of his experiments in the production of milk in winter under free versus restricted ventilation, suggests as follows: "Milk produced in a building kept at a high temperature, or during a warm period, does not seem to be any richer in fat than that produced at a low temperature or during cold weather."

Hays[3] has shown that he obtained an increase of approximately 0·2 per cent. of fat in cows' milk for each 10° lowering of the temperature within the limits of 70° and 30° F. He concludes that temperature is a major factor in the seasonal variation of the percentage of fat in cows' milk.

Weaver and Matthews[4], working with Ayrshire, Holstein, Guernsey and Jersey cattle, concluded that butterfat tests were lower with higher outside and inside temperatures. They add, however, that as measured by regression coefficients, butterfat tests were affected more by changes in environmental temperature than by the other factors studied, and further, that although it was not determined whether variations in outside or inside temperature had the greater influence upon the fat test, there were indications that variation in the fat test were more closely related to variations in outside temperature.

This is one of the few pieces of work that appears to consider the possible effect of variation of temperature on fat tests, but it is not made

very clear whether the temperature variation referred to is a daily, weekly, or seasonal variation. Further, the value of the work is to some extent lost owing to the use of average figures for morning and evening milk.

The problem facing research students in this country is: "Why is morning milk under customary twice-daily milking conditions so frequently below presumptive standard in the case of certain breeds?" This question can only be answered satisfactorily by studying the milk of individual cows at each of the two or three milkings separately.

The writer(5), in the course of a thrice-daily milking trial started in 1927, took maximum and minimum temperatures twice daily both inside the cowhouse and outside. Thermometer readings were made at 7 a.m. and 10 p.m.

A total of 10,689 samples from individual cows was tested for fat and of this number 1262 or 11·8 per cent. fell to or below 2·95 per cent. of fat. The low fats were produced per milking as follows:

6 a.m.	...	...	38·9	per cent.
2 p.m.	...	...	34·4	,,
10 p.m.	...	...	26·7	,,

In view of the fact that the cows in this trial were milked at three 8-hour intervals, it became necessary to look for factors other than the interval factor to account for these low fats, particularly in view of the very close agreement between the percentage of low fats in this trial and the percentage commonly found on the farm where twice daily milking at uneven intervals is practised. Following the above-mentioned trial, two cows were milked for the first 27 weeks of their lactations, at intervals of 16 and 8 hours for the first 15 weeks, and of $14\frac{1}{2}$ and $9\frac{1}{2}$ hours for the remaining 12 weeks. The long interval in each case, however, was from morning to night, not night to morning.

The results indicated much need for further research along these lines, and the work is being repeated during the present winter (1930–1) with a larger number of cows. Briefly, these two cows gave milk in quantities at each milking which bore a different relationship, the one to the other, than is usually the case when the same intervals apply in ordinary farm practice, but where the long interval is from night to morning. The same applied to the fat percentage. Partly as a result of this work we found reason to suggest that night itself, factors operating at night or the interrelationship of day and night factors, were material in bringing about low fat percentages in the milk of individual cows, and that the long night

interval under customary milking conditions merely served to concentrate the low fats in bulk morning milk.

OBJECT.

The main object of this work, herein reported, was therefore to investigate daily fluctuations of temperature in order to find out if there was any connection between high daily ranges of temperature in any one week or month and the number of low fats produced. Persistent low fat percentage has seldom been remarked as a concomitant result of persistent low temperature in the winter. Attention is drawn particularly to the summer period—marked on Fig. 1—when the herd was out day and
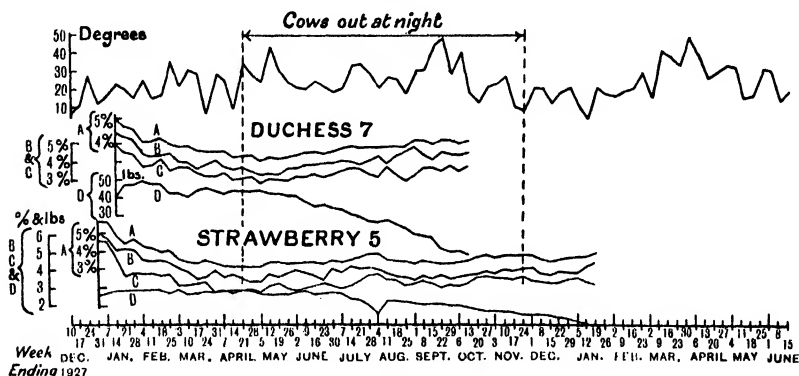
Fig. 1.

night and, therefore, exposed to the full effect of temperature fluctuation. By temperature fluctuation and daily range of temperature is meant the difference between the maximum day temperature and the minimum night temperature.

DESCRIPTIVE.

From the maximum and minimum temperatures recorded outside the cowhouse from December 4, 1927, to June 15, 1929, the daily range of temperature was calculated.

These outside daily ranges of temperature were averaged to show the average range for each week of the whole period, and again for each calendar month of 12 months of the period.

Table I shows the average daily range outside per month from January 1928 to December 1928 and the percentage number of low fats that occurred in each month—that is, fats at or below 2·95 per cent. The

average daily range of temperature per week is shown in the curves in Fig. 1.

Table I.

	Average daily range per month (° F.)	Percentage of low fats	
January	19	5·0	
February	23	2·1	
March	24	3·3	
April	24	17·5	Total number of
May	30	18·5	samples tested
June	21	16·6	per day remained
July	29	14·7	constant for 5
August	24	13·5	months ending
September	39	16·5	September 16th
October	22	11·1	
November	19	7·2	
December	18	11·8	

It is worthy of note that in 1928 the maximum range in the first 5 months occurs in May with the maximum percentage of low fats. The increased percentage of low fats in April as compared with March is referred to later.

It must also be noted that the maximum range of temperature for the year occurred in September, and that this maximum range coincides with an increased percentage of low fats over the preceding month—this in spite of the fact that of the twelve cows in milk, three were finally dried off in September, two in October, and three in November, at which period of lactation fat percentage tends to be variable, but high.

The average daily range of temperature outside per week has been plotted on each of the Figs. 1 to 7. The curve in Fig. 1 covers the whole period of 18 months, but the range curve in the remaining figures covers only the period or periods relevant to the remaining curves.

The following will explain the origin of the remaining curves: from the total weight of milk and fat per week in the case of each cow, the true fat percentage for that week was calculated. This constitutes curve *A* for each cow.

From the total weight of milk and fat at each of the three milkings was calculated the true fat percentage for each of the three milkings separately for the week.

Curve *B* represents the highest of these fat percentages, curve *C* the lowest—regardless in each case of the milking at which each occurred. For example, Fig. 1, Duchess 7: this cow has her highest true fat percentage per milking at the 10 p.m. milking in one week, possibly at the 2 p.m. in a following week. Both are plotted in curve *B*. Her lowest
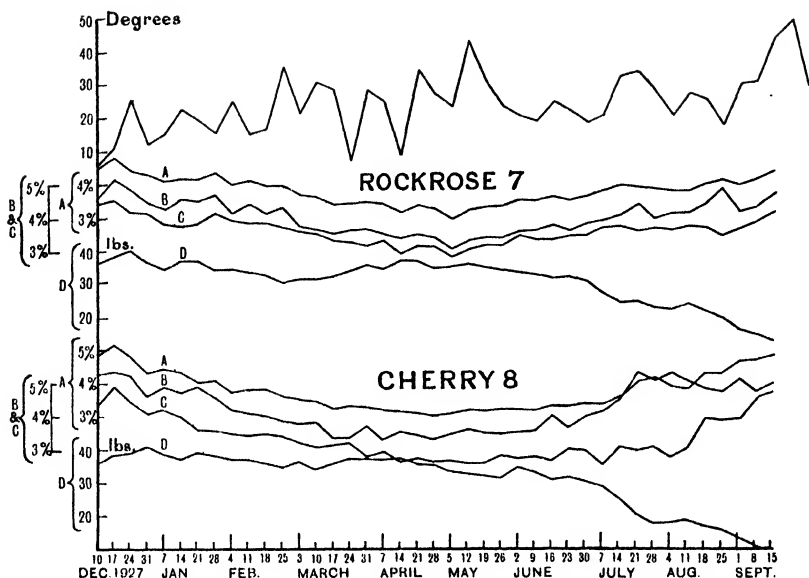
Fig. 2.
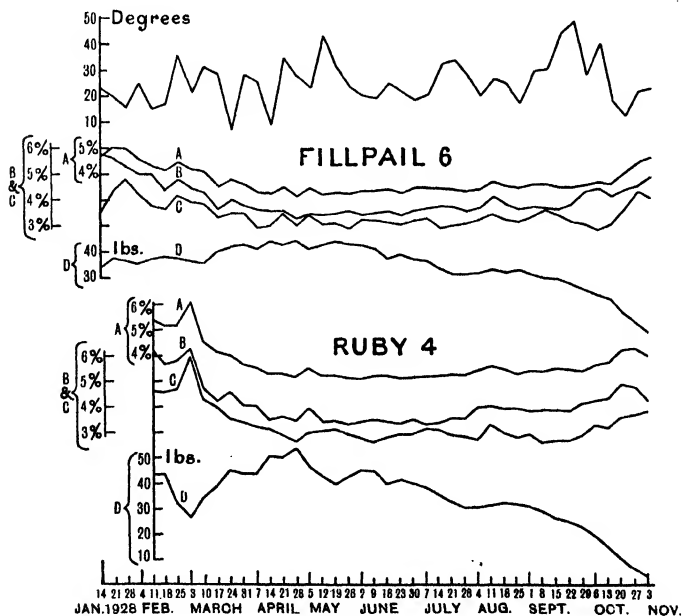
Fig. 3.

occurred at the 6 a.m. milking one week, possibly at the 10 p.m. or 2 p.m. in a following week. Both are plotted in curve *C*.

Curve *D* represents the daily average yield of milk per cow per week.

Weekly averages of daily temperature range and the fat percentages for periods of a week have been used in this work in preference to actual daily ranges of temperature and actual daily fat percentages. Assuming, for the sake of argument, that temperature has some effect on milk production, it seems unlikely that each individual cow will be affected in precisely the same time following a big rise or fall in temperature.
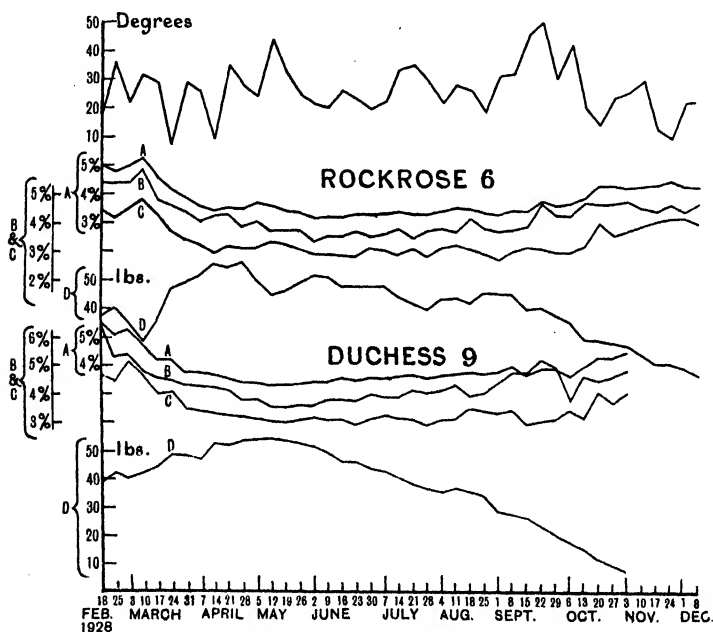
Fig. 4.

Actually, when an attempt was made to examine daily ranges of temperature together with daily fat percentages, some cows appeared to show a lag in their assumed responses to temperature variation. In order to eliminate to some extent the possible affect of lag, average figures have been used, the week being selected as the time unit. There is some justification for the view that lag plays a part in this matter of temperature and its assumed effect on milk production. Some cows show a very marked fluctuation in milk yield and fat percentage during the 24 hours

before actually showing oestrum symptoms, some during the same 24 hours, and others in the 24 hours following.

Although Table I shows average temperature range per month for a whole year, the curves (Figs. 1 to 7) for individual cows should be studied largely for the period during which the cows were outside both day and night, because it is chiefly during this period that the cows were really exposed to wide variations of temperature.
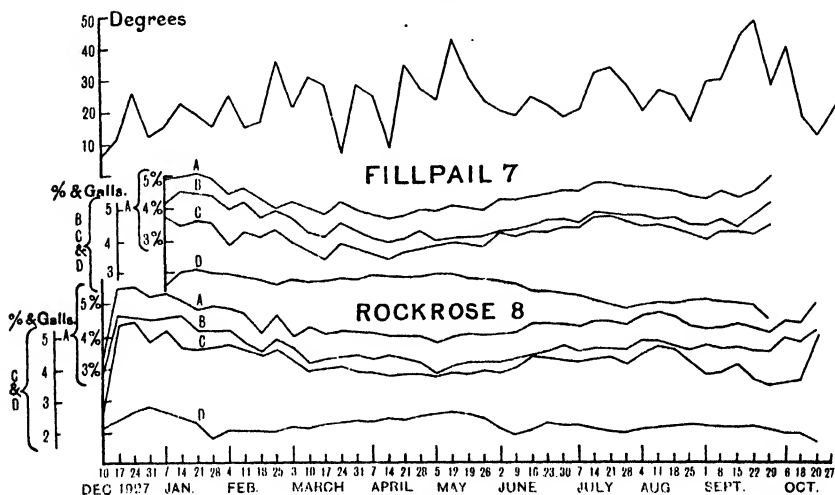
Fig. 5.

The average temperature range of March and April was the same, but the percentage number of low fats rises from 3·3 in March to 17·5 in April. It has not been forgotten that other factors may have contributed to this increase, but it is suggested that temperature range is probably the chief factor. Of the total number of low fats found in April, 70 per cent. occurred in the second half, that is, during that part of the month when most of the cows were out day and night, and most of the cows showed no increase in milk yield in the second half of the month to account for the increased number of low fats over the first half of the month. The average daily range of temperature for the first 15 days of April was 16·5°, and for the second 15 days, 32·25°.

OBSERVATIONS.

A study of the curves accompanying this report shows:

(1) That there is frequently a depression of milk yield occurring during a period of wide average temperature range.
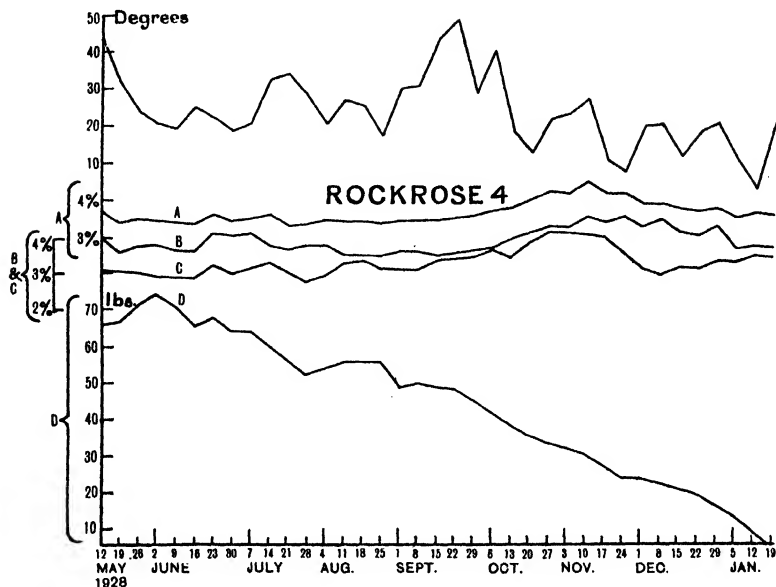
Fig. 6.
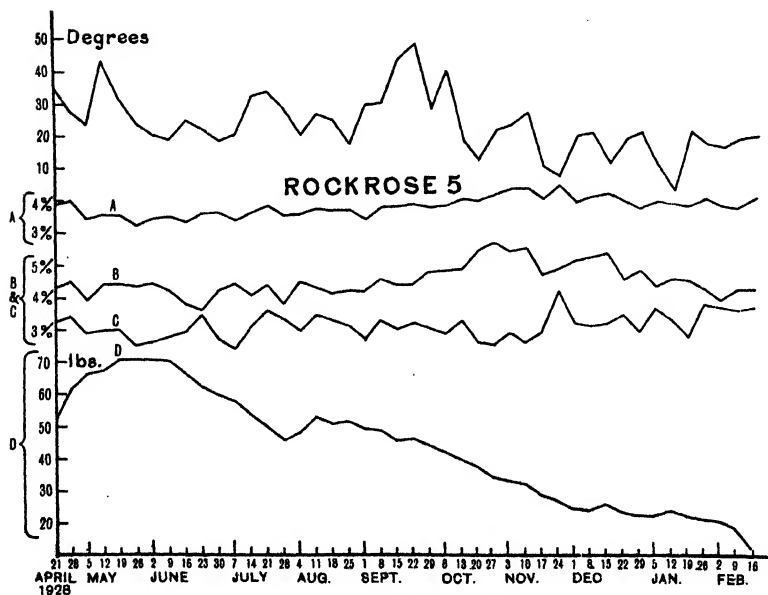
Fig. 7.

(2) That there is marked variation of the fat percentage curves *B* and *C*, highest and lowest fat percentage curves respectively. That this variation is more marked in curve *C* than in curve *B*, and that frequent depression of curve *C* occurs during a period of wide average temperature range. Notice particularly curve *C* of Rockrose 5.

It is suggested that the above-mentioned depressions and variations are due in large part to extreme average temperature range, except in two cases, as follows: Rockrose 6 and Ruby 4 developed very severe chills just after calving and consequently dropped considerably in milk yield.

Before arriving at the above conclusion, attention was paid to the following as being factors which might have contributed to the results which have been attributed to temperature variation:

(*a*) Change in quantity and type of food.

(*b*) Change from one pasture to another.

(*c*) Excess, lack of, or variable amount of exercise.

(*d*) Lack of drinking water.

(*e*) Unusual distribution of rainfall.

It is stated with some degree of confidence that the influence on milk yields or fat percentages of changes under (*a*) and (*b*) was not such as to make itself obvious when average figures are used with the week as the time unit.

The amount of compulsory exercise was constant from day to day throughout the winter, and from day to day throughout the summer.

There is no lack of drinking water outside and water bowls are fitted in the cowhouses.

The rainfall for the year was only 0·9 inch in excess of the average for the preceding 10 years, and the distribution of rain per month of the year was normal. As far as the change from winter to summer conditions is concerned the following is of interest: four cows in the trial, Strawberry 5, Cherry 8, Ruby 4, and Rockrose 6 were kept in under winter conditions until the week ending May 26. In order to show the daily range of temperature to which these four cows were exposed the difference between the maximum temperature outside during the day and the minimum temperature inside during the night has been calculated. The figures are shown in Table II beside figures for the same weeks showing the difference between maximum and minimum temperatures outside.

A comparison of the curves of this group of cows during this period and later with the other cows during the same period and later appears to

suggest that some common factor was operating to influence yields and fat percentages.

<p align="center">Table II.</p>

Week ending	Average daily range of temperature per week as between outside and inside	Average daily range per week outside
April 28	18	28
May 5	18	24
„ 12	36	44
„ 19	25	31
„ 26	24	24

The point that stands out clearly is that the one environmental factor during the whole summer period that was least constant was temperature, and it is suggested that it is this lack of constancy in temperature, this wide variation as between day and night temperature, which was primarily responsible for fluctuating milk yields and fluctuating and low fat percentages.

<p align="center">SUMMARY AND CONCLUSIONS.</p>

1. Curves are given to show:

(*a*) Weekly averages of daily temperature range over a period of eighteen months.

(*b*) The true fat percentage per week of all three milkings together in the case of each cow for her full lactation (curve *A*).

(*c*) The highest and lowest true fat percentage per week in the case of each cow (curves *B* and *C* respectively).

(*d*) Weekly averages of daily milk yield in the case of each cow (curve *D*).

These curves show that at certain periods there are marked depressions of milk yield and fat percentage and marked tendencies to variation of fat percentage.

2. An attempt has been made to show that there is frequent association between wide temperature range on the one side and depression of milk yield, and, for at any rate one or two milkings of the three per day, depression of fat percentage on the other. Further, it is suggested that appreciable variation of the minimum fat percentage is noticeable during periods of wide temperature range.

Consideration has been given to other factors as a cause of these depressions and variations, and it is considered that there is no evidence to show that these factors influenced the results to any noticeable extent. It is suggested, however, that there are indications that temperature

variation has shown itself to be one, if not the chief, cause of the fluctuations of milk yield and fat percentage.

The opinion is expressed that temperature variation per 24 hours is a fruitful cause of morning milk being low in fat under twice daily milking conditions with uneven intervals.

I wish to express my appreciation of the assistance given by Prof. Pennington, Prof. Neville, Captain Golding, and Mr Mackintosh in the construction of this report.

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A UNIFORMITY TRIAL WITH IRRIGATED BROADCAST RICE.

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(With One Text-figure.)

A STUDY of the technique of comparative field trials with pedigree rice selections in Ceylon (1) had shown the desirability of conducting an *ad hoc* experiment to determine (*a*) the optimum size of plot for field trials with rice, and (*b*) the effect on yield of the position of the plot in the field. In the early years of the life of a new selection only small quantities of seed are available and this necessitates laying down yield trials in small plots. After 3 or 4 years the seed available is usually sufficient to enable this factor to be disregarded in determining plot size. Plot size, however, is sometimes determined by another factor outside the control of the experimentalist, that is, by the size of the fields. In some districts of Ceylon the small pocket-handkerchief fields—found generally where there is terraced cultivation on hillsides—entirely preclude the laying down of field trials. And in manurial experiments with rice which entail separate bunded plots for each manurial treatment, a plot larger than the theoretical optimum may be found more convenient. Excluding these two factors of seed supply and smallness of fields plot size is determined in practice by (*a*) the standard errors of plots of different sizes, and (*b*) their convenience for the different agricultural and other operations necessary.

The effect of proximity to bunds in increasing yields has been widely noticed and different methods have been used to prevent this from unduly influencing experimental results. The uniformity trial here described clearly shows this influence of bunds on yield and also the effect of placing long, narrow plots both at right-angles to and parallel with the irrigation channel.

DESCRIPTION OF THE EXPERIMENT.

In 1929 eight 1/5 acre fields at the Anuradhapura Experiment Station in the northern dry zone of Ceylon were broadcast with the local rice at the rate of 70 lb. per acre. The variety of rice grown, *vellai illankalayan*, matures in four months and wherever rices of this or lesser age are grown in Ceylon transplanting is not done. Shortly after broadcasting each field

was marked out into seventy 10 ft. × 10 ft. plots by ropes which were pegged down and which remained in position until after harvesting. Fig. 1 is a plan of one of the fields. Between the outside plots and the bunds was a border varying from 1 ft. to 3 ft. wide; the borders were completely
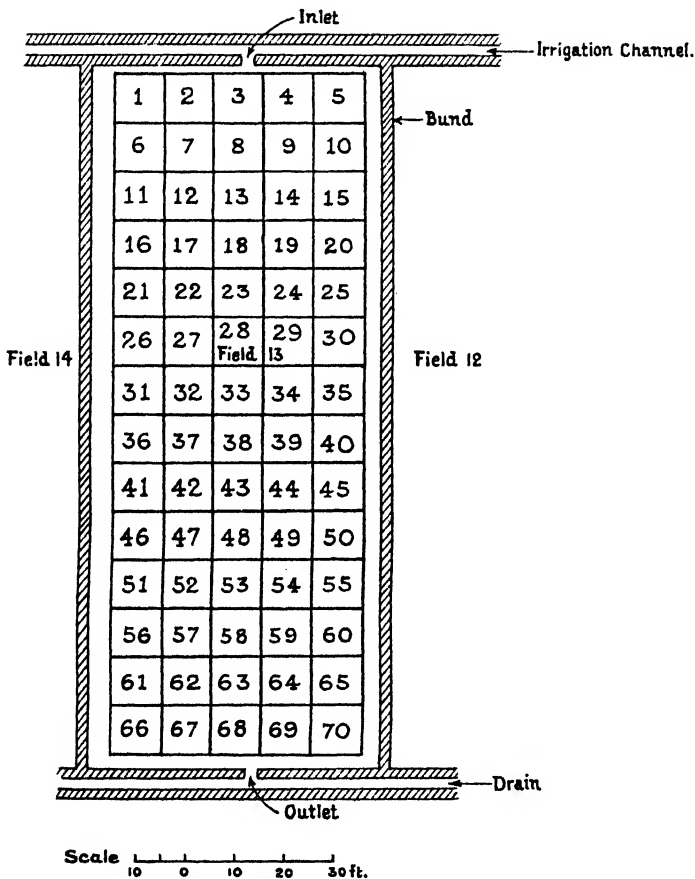
Fig. 1.

occupied by plants (as the whole area of each field was broadcast) and this eliminated any border effect. There was no space between plots. Each of the 560 plots was harvested separately and threshed by a hand-threshing machine. Weights were recorded after the grain and straw had been thoroughly sun dried. Plots of the desired sizes were built up from these units and with all plots used in the calculation of standard devia-

tions in each field the five plots 1–5 along the irrigation channel, and the five plots 66–70 along the drain were excluded owing to the better growth of these plots. The calculation of standard deviations was made by the method described by R. A. Fisher[2]. The different fields were used as blocks for the elimination of inter-block, or here, inter-field, variance. As will be seen from the results the yield variation between different bunded fields is large, and it is not considered justifiable to allow this to swell the remainder variance. In all field trials with swamp rice any replication should be contained within one bunded field and, even where Latin squares are used, *either* each row *or* each column should be laid down in this manner, and if the whole square can be completed in one field so much the better.

<div align="center">Statement of results.</div>

The grain and straw yields of the 10 ft. × 10 ft. plots will be found in the appendix to this paper. The total yields of the eight fields are as follows:

	Weight in lb.	
Field no.	Grain	Straw
10	589·5	732·2
11	501·7	599·7
12	314·6	488·0
13	290·6	538·3
14	489·3	670·0
26	441·3	559·8
27	451·0	629·4
28	530·2	718·4

The importance of eliminating inter-field variance is obvious. Fields 10–14 were adjacent fields on one side of the drain and fields 26–28 adjacent fields on the other side.

Tables I and II are attempts to demonstrate the influence of bunds, irrigation channels and drains on the yields of the plots adjoining these. Table I gives the mean yields of the unit plots of 10 ft. × 10 ft. for the different fields in columns, that is to say, the mean yields of the fourteen plots 1, 6, 11, 16, etc., to 66 are shown for the eight fields in the column headed 1–66. Similarly the mean yields of the fourteen plots 2, 7, 12, 17, etc., to 67 are shown in the column headed 2–67, and so on. It will be seen that the plots adjoining the bunds have yielded much higher than the rest. The plots in the middle column (*i.e.* the middle of the field) have yielded the poorest. Table II is a similar table showing the mean yields of the rows of plots across the fields. In the column headed 1–5 are the mean yields in the eight fields of the five plots 1, 2, 3, 4 and 5. Under the

heading 6–10 are the mean yields for each field of the five plots 6, 7, 8, 9 and 10, and so on. Again it will be seen that the plots adjoining the irrigation channel and the drain have given the highest yields. To eliminate the columns of plots at the sides of the fields, *i.e.* plots 1, 6, 11, etc., to 66 and 5, 10, 15, etc., to 70 would seriously curtail the available experimental area of the fields so it was decided to eliminate in all the fields the row of plots along the irrigation channel (plots 1, 2, 3, 4, 5) and that along the drain (plots 66, 67, 68, 69, 70). The mean yields of these two rows are respectively 16 and 14 per cent. (grain) and 31 and 13 per cent. (straw) greater than the yields of adjoining rows.

Table I. *Mean yield of fourteen 10 ft. × 10 ft. plots in columns.*
(*Plots at right-angles to irrigation channel.*)

Weight of grain in lb.

Field no.	1–66	2–67	3–68	4–69	5–70
			Plots		
10	9·03	8·08	8·39	8·20	8·41
11	7·42	6·87	6·99	7·05	7·50
12	5·74	3·24	2·59	4·13	6·77
13	4·74	3·37	3·67	4·10	4·88
14	7·53	6·99	6·13	7·02	7·28
26	7·82	6·21	5·82	5·49	6·19
27	6·79	6·24	5·94	6·19	7·05
28	8·60	7·76	6·10	7·42	7·99
Total...	57·67	48·76	45·63	49·60	56·07
Mean...	7·208	6·095	5·703	6·200	7·008

Weight of straw in lb.

10	11·04	9·86	9·80	10·51	11·08
11	8·94	8·17	8·39	8·29	9·06
12	7·20	6·39	5·66	6·86	8·75
13	7·94	6·93	7·75	7·41	8·41
14	9·41	8·86	7·88	10·31	11·40
26	9·97	7·79	7·56	6·86	7·79
27	9·16	8·89	8·78	8·31	9·83
28	11·66	10·56	8·09	10·66	10·34
Total...	75·32	67·45	63·91	69·21	76·66
Mean...	9·415	8·431	7·988	8·651	9·582

Table III gives the standard deviations of different sized plots and Table IV the analyses of variance for the 1/436 acre plots.

It will be seen that the standard deviations of the 1/87 acre plots at right-angles to the irrigation channel are much higher than those of the same sized plots parallel to it. It does not follow that this would be so under different conditions.

The efficiency of plots of different sizes may be judged by comparing the standard error of a particular plot with that of the mean of the num-

Table II. *Mean yield of five 10 ft. × 10 ft. plots in rows.*
(Plots parallel to irrigation channel.)

Weight of grain in lb.

Field no.	Plots													
	1–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	66–70
10	8·66	8·62	8·00	8·22	7·90	8·36	8·42	8·14	8·34	8·12	8·78	8·54	9·04	8·76
11	6·70	5·48	6·40	6·52	6·86	7·22	7·60	7·76	7·56	7·30	7·58	7·48	7·98	7·90
12	6·54	4·24	4·54	4·48	3·80	3·72	3·30	3·88	4·22	4·26	4·44	4·22	4·76	6·52
13	4·22	3·22	3·58	3·70	4·06	3·14	2·76	3·46	5·30	5·66	3·70	3·98	4·50	6·84
14	7·52	6·14	6·34	6·80	6·70	7·18	6·96	7·10	6·78	7·04	7·02	6·74	7·40	8·14
26	8·62	7·48	7·36	6·98	5·62	5·58	5·34	5·74	4·80	4·94	5·92	6·52	6·58	6·78
27	6·62	5·76	6·44	6·96	7·24	6·72	6·60	6·72	6·92	6·50	6·38	6·06	4·86	6·42
28	8·04	7·98	7·38	7·80	7·98	8·16	8·02	7·54	7·64	7·44	6·98	7·08	6·58	7·42
Total...	56·92	48·92	50·04	51·46	50·16	50·08	49·00	50·34	51·56	51·26	50·80	50·62	51·70	58·78
Mean...	7·115	6·115	6·255	6·432	6·270	6·260	6·125	6·292	6·445	6·407	6·350	6·327	6·462	7·347

Weight of straw in lb.

Field no.	1–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	66–70
10	12·12	11·04	10·08	10·14	10·18	9·84	9·78	10·78	10·08	10·16	10·14	10·64	10·28	11·18
11	8·64	7·00	7·10	7·44	8·18	8·30	8·48	9·74	9·64	7·54	8·80	9·64	9·64	9·80
12	8·78	6·02	6·76	5·98	5·34	5·28	5·32	6·94	6·80	7·72	6·84	8·04	8·54	9·24
13	8·52	6·24	6·88	6·18	7·34	6·96	6·58	7·46	8·74	9·64	7·10	6·64	8·90	10·48
14	11·26	7·18	8·64	8·60	8·68	10·54	8·64	9·08	7·98	10·02	10·88	10·00	9·90	12·60
26	12·04	9·00	9·30	8·58	7·32	7·34	6·74	8·04	6·04	6·24	7·10	8·34	7·14	8·74
27	11·48	9·26	9·34	9·02	10·08	10·04	8·02	8·70	9·34	8·44	8·58	9·08	6·60	7·90
28	12·34	9·38	10·72	10·22	11·60	11·24	10·30	10·04	10·14	9·40	9·50	9·60	9·60	9·60
Total...	85·18	65·12	68·82	66·16	68·72	69·54	63·86	70·78	68·76	69·16	68·94	71·98	70·60	79·54
Mean...	10·647	8·140	8·602	8·270	8·590	8·692	7·982	8·847	8·595	8·645	8·617	8·997	8·825	9·942

ber of units used to make up the plot. For example it requires five 1/436 acre plots to make up one 1/87 acre plot. The standard deviation of a 1/87 acre plot (parallel to the irrigation channel) is 9·8 per cent. The standard deviation of the mean of five 1/436 acre plots is 20·1 per cent. $\div \sqrt{5} =$ 8·99 per cent. Table V shows this comparison for the four sizes of plots for grain.

Table III. *The standard deviations of plots of different sizes.*

| Size of plot | Degrees of freedom | s.d. as percentage of mean | | Remarks |
		Grain	Straw	
10 ft. × 10 ft. = 1/436 acre	472	20·1	19·7	Plots parallel to irrigation channel
10 ft. × 50 ft. = 1/87 acre	88	9·8	11·2	„ „
10 ft. × 50 ft. = 1/87 acre	72	16·7	15·0	Plots at right-angles to irrigation channel
20 ft. × 50 ft. = 1/44 acre	40	8·8	9·6	Plots parallel to irrigation channel
40 ft. × 50 ft. = 1/22 acre	16	7·8	8·9	„ „

Table IV. *Analyses of variance, 1/436 acre plots.*

Grain.

Variance	Degrees of freedom	Sum of squares	Mean square	s.d.	s.d. as percentage of mean
Total	479	1792·7472	—	—	—
Fields (or blocks)	7	1031·9234	—	—	—
Remainder	472	760·8238	1·61	1·2688	20·1

Straw.

Total	479	2061·2937	—	—	—
Fields (or blocks)	7	714·9417	—	—	—
Remainder	472	1346·3520	2·85	1·688	19·7

Table V. *Actual and theoretical reduction of the standard deviation on increasing size of plot.*

Grain.

| Size of plot (acre) | s.d. as percentage of mean | |
	Actual	Theoretical
1/436	20·1	—
1/87	9·8	8·99
1/44	8·8	6·36
1/22	7·8	4·49

The opportunity was taken of examining the efficiency of Latin squares extending over more than one bunded field. It ·had been thought that unless a Latin square was complete in one bunded field (and this would seldom be possible in Ceylon except with very small plots) the amount

of the extra variance eliminated would not compensate for the loss in degrees of freedom.

Two groups of three adjacent fields, each group irrigated from the same channel, were used and each field was divided into three plots of 1/22 acre. Each column, therefore, was complete in one field while the rows extended over three fields. Table VI shows the standard deviations of the Latin squares and of the same plots without the elimination of the "row" variance.

Table VI. *A comparison between blocks and Latin squares.*

Size of plot	Degrees of freedom	S.D. as percentage of mean		Remarks
		Grain	Straw	
1/22 acre (40 ft. × 50 ft.)	4	6·7	8·2	Latin square, fields 10–12
,, ,,	6	7·2	9·2	Blocks, fields 10–12
,, ,,	4	7·6	4·6	Latin square, fields 26–28
,, ,,	6	8·4	7·6	Blocks, fields 26–28

DISCUSSION OF THE RESULTS.

The list of the total yields of the eight fields is a striking lesson in the necessity for correctly laying down field experiments. The yield of grain in field 14 is 68 per cent. greater than that of the adjoining field 13 under similar conditions of cultivation and treatment. It is extremely unfortunate that the large inter-field variation with rice which is frequently met with is not always realised, and that even now trials are carried out which ignore this. From Tables I and II it will be seen that the intrafield variation is also very large; yields increase as the bunds are approached. Why this should be so is not clear. The phenomenon has been noticed in Malaya. In Upper Burma [3] the yields of plots next to the irrigation channel and drains were higher than normal, but the yields of plots next to ordinary bunds were lower. The writer has lately been informed that, in Lower Burma, yields of plots adjoining bunds are also below normal. The varied conditions under which rice is grown in the Tropics and the different methods of cultivation render it impossible to draw general conclusions from one trial. If the increase in yield had been found only on two opposite sides of the fields, it would have been possible to lay out plots along the fertility gradient and so eliminate its effect in the calculation of variance. Unfortunately there is a fertility gradient (in the shape of a flattened V) in both directions. It was decided, therefore, that the most practical method of dealing with this increase of yield near the bunds was to exclude plots 1, 2, 3, 4 and 5 adjoining the irriga-

tion channel, and plots 66, 67, 68, 69 and 70 adjoining the drain and then to form plots which would run across the whole field. Had the fields been square, or if the long axis of the rectangle had run alongside the irrigation channel, it would doubtless have been more effective to lay out plots at right-angles to the irrigation channel. In almost square fields in Burma[3] it was found that plots at right-angles to the irrigation channel had a definitely smaller standard deviation than those parallel to it. A factor largely accounting for this was the presence of large quantities of silt in the irrigation water. Little or no silt is carried by the irrigation water at Anuradhapura where the present trials were conducted.

From Table III it will be seen that the standard deviations even with plots of 1/22 acre are high, and increasing the size of the plots above 1/87 acre has had little effect in reducing them. The standard deviation of the unit plot of 1/436 acre is 20 per cent., of the 1/87 acre plot 9·8 per cent., of the 1/44 acre plot 8·8 per cent., and of the 1/22 acre plot 7·8 per cent.

From Table V it is seen that the reduction of the variance on increasing the size of the plot varies widely from the theoretical rate of reduction at least with plots larger than 1/87 acre. It would appear that plots of about 1/87 acre are the most effective. Smaller plots with a comparatively slightly lower variance would have to be replicated so many times to reduce errors to a reasonable size that their use would be costly and in-convenient. To reduce the standard error of the difference between two means to about 5 per cent. would require thirty-two replications of 1/436 acre plots ($20·1 \times \sqrt{2/32} = 5·02$), eight replications of 1/87 acre plots ($9·8 \times \sqrt{2/8} = 4·90$), six replications of 1/44 acre plots ($8·8 \times \sqrt{2/6} = 5·08$) and five replications of 1/22 acre plots ($7·8 \times \sqrt{2/5} = 4·93$). Where space is not limited the use of 1/44 and 1/22 acre plots will save replications but will require a much larger area of land and will be more costly. Taking everything into consideration, plots of about 1/87 acre will probably be found to be the most practicable. As a matter of interest it may be stated that 1/100 acre plots of transplanted rice have in Ceylon given standard deviations varying from 9·5 to 14·8 per cent.

In the comparison of plots laid down in Latin squares and in randomised blocks it is seen in Table VI that the elimination of the extra variance in the Latin squares has resulted in smaller standard deviations. The value of t (see Fisher[2]), however, is so much greater when $n = 4$ than when $n = 6$ that the reduction in the error is counterbalanced by the reduction in the available degrees of freedom (except in the error for straw weights in fields 26–28; the great reduction in this instance is not understood), and there has been no net gain by the use of the Latin square method of

laying down plots. This comparison applies only to a Latin square which covers more than one bunded field and, as has been mentioned before, fields in Ceylon are so small that it will seldom be possible to have a Latin square complete within one bunded field.

SUMMARY.

This paper describes a uniformity trial with irrigated broadcast rice and shows the large inter- and intra-field variation which exists.

The standard deviations decrease with the increased size of plots and vary from 20·1 per cent. with 1/436 acre plots to 7·8 per cent. with 1/22 acre plots.

The decrease of the standard deviation is small with plots larger than 1/87 acre. This size of plot is considered to be the most practicable for field trials.

The influence of proximity to bunds, irrigation channels and drains on increasing yields is discussed and methods of reducing errors due to this cause are suggested.

It is shown that the use of a Latin square extending over more than one field has not produced a more accurate experiment than the use of randomised blocks. The latter method would therefore appear to be the more suitable for manurial and other trials, under the particular conditions of rice growing.

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APPENDIX.

Yield in lb. of 10 ft. × 10 *ft. plots.*

Yields of straw in italics.
The arrangement of the plots is as in Fig. 1.

	Field 10						Field 11			
	9·2	8·4	8·5	9·2	8·0	5·4	6·8	7·4	6·8	7·1
	12·2	*11·7*	*12·5*	*12·0*	*12·2*	*7·0*	*9·0*	*10·0*	*7·5*	*9·7*
	9·8	8·2	8·3	8·2	8·6	4·7	5·8	5·2	4·8	6·9
	12·0	*11·5*	*10·2*	*10·0*	*11·5*	*6·0*	*7·0*	*8·5*	*5·5*	*8·0*
	8·3	8·2	7·2	8·6	7·7	7·2	6·8	5·8	6·7	5·5
	10·0	*9·7*	*9·2*	*10·5*	*11·0*	*7·5*	*7·0*	*7·0*	*7·5*	*6·5*
	7·5	8·4	8·3	8·5	8·4	6·4	6·7	6·9	6·7	5·9
	11·0	*9·2*	*9·0*	*10·0*	*11·5*	*9·0*	*7·0*	*6·7*	*8·0·*	*6·5*
	8·7	7·4	8·2	7·3	7·9	6·7	7·2	6·4	6·9	7·1
	10·0	*10·0*	*9·7*	*10·2*	*11·0*	*7·7*	*9·0*	*7·5*	*8·5*	*8·2*
	9·3	8·5	7·9	7·8	8·3	7·3	6·9	6·5	7·6	7·8
	11·0	*9·5*	*8·5*	*10·0*	*10·2*	*8·0*	*7·0*	*7·5*	*9·0*	*10·0*
	9·1	8·7	8·0	7·7	8·6	8·2	6·8	7·2	8·1	7·7
	10·5	*9·2*	*9·5*	*9·7*	*10·0*	*9·0*	*7·2*	*8·5*	*9·0*	*8·7*
	8·4	7·8	7·9	8·2	8·4	8·3	6·8	7·9	7·6	8·2
	11·0	*9·7*	*9·7*	*11·5*	*12·0*	*11·5*	*8·2*	*8·5*	*11·0*	*9·5*
	9·3	6·8	8·6	8·3	8·7	7·6	6·9	8·0	7·3	8·0
	10·5	*8·2*	*9·2*	*11·0*	*11·5*	*12·0*	*8·5*	*9·5*	*9·0*	*9·2*
	9·2	6·3	8·6	7·8	8·7	8·8	6·7	6·5	6·4	8·1
	10·2	*8·5*	*9·7*	*10·2*	*12·2*	*7·5*	*7·0*	*6·7*	*8·0*	*8·5*
	9·9	8·2	8·7	8·3	8·8	8·2	6·9	7·8	6·0	9·0
	10·5	*10·0*	*10·5*	*9·7*	*10·0*	*10·0*	*8·5*	*8·5*	*6·5*	*10·5*
	9·2	8·3	8·7	8·4	8·1	8·5	7·0	6·7	7·5	7·7
	11·5	*9·7*	*9·0*	*12·5*	*10·5*	*9·7*	*11·0*	*8·0*	*8·5*	*11·0*
	8·9	9·2	9·4	8·6	9·1	8·3	7·5	7·7	8·0	8·4
	13·7	*10·0*	*8·5*	*8·7*	*10·5*	*10·2*	*9·0*	*9·5*	*9·0*	*10·5*
	9·6	8·7	9·1	7·9	8·5	8·3	7·4	7·9	8·3	7·6
	10·5	*11·2*	*11·2*	*11·2*	*11·0*	*10·0*	*9·0*	*11·0*	*9·0*	*10·0*
Total ...	126·4	113·1	117·4	114·8	117·8	103·9	96·2	97·9	98·7	105·0
	154·6	*138·1*	*137·2*	*147·2*	*155·1*	*125·1*	*114·4*	*117·4*	*116·0*	*126·8*
Mean ...	9·03	8·08	8·39	8·20	8·41	7·42	6·87	6·99	7·05	7·50
	11·04	*9·86*	*9·80*	*10·51*	*11·08*	*8·94*	*8·17*	*8·39*	*8·29*	*9·06*

	Field 12						Field 13			
	7·2	5·4	7·1	6·2	6·8	4·5	4·5	4·7	4·2	3·2
	8·2	*8·0*	*9·5*	*8·2*	*10·0*	*8·5*	*9·2*	*9·2*	*8·7*	*7·0*
	5·1	3·1	3·9	4·1	5·0	3·6	1·1	2·9	1·6	6·9
	6·2	*4·7*	*5·5*	*4·7*	*9·0*	*4·7*	*5·0*	*5·0*	*7·5*	*9·0*
	5·6	3·8	1·8	4·9	6·6	5·2	1·9	1·8	3·7	5·3
	7·7	*6·2*	*4·2*	*6·5*	*9·2*	*10·7*	*5·7*	*4·5*	*7·0*	*6·5*
	5·7	3·3	1·5	4·9	7·0	5·1	2·8	4·6	4·1	1·9
	8·0	*4·5*	*4·0*	*5·7*	*7·7*	*7·5*	*4·2*	*7·2*	*7·0*	*5·0*
	4·7	3·3	1·2	3·0	6·8	6·7	2·3	2·4	4·2	4·7
	6·0	*5·7*	*2·5*	*4·5*	*8·0*	*10·5*	*5·0*	*5·2*	*7·0*	*9·0*
	5·2	2·4	1·9	2·4	6·7	4·4	2·3	3·3	2·7	3·0
	5·5	*5·2*	*4·0*	*4·5*	*7·2*	*8·2*	*5·7*	*7·7*	*5·2*	*8·0*
	4·8	2·7	1·1	2·2	5·7	4·0	1·7	2·9	2·6	2·6
	6·2	*5·7*	*4·0*	*4·5*	*6·2*	*6·2*	*5·0*	*9·0*	*5·0*	*7·7*
	8·7	2·5	1·1	1·9	5·2	4·3	3·3	2·6	3·1	4·0
	11·2	*6·0*	*4·5*	*5·5*	*7·5*	*7·2*	*6·2*	*11·0*	*5·7*	*7·2*
	6·6	2·8	1·8	4·2	5·7	5·2	4·4	4·6	6·1	6·2
	7·2	*7·2*	*6·2*	*6·7*	*6·7*	*8·5*	*8·0*	*8·0*	*8·0*	*11·2*
	5·5	3·4	1·9	3·1	7·4	4·6	6·2	4·2	5·9	7·4
	5·7	*8·0*	*4·7*	*9·2*	*11·0*	*8·5*	*11·0*	*9·0*	*9·0*	*10·7*
	6·1	2·8	1·8	3·5	8·0	3·7	3·2	2·7	4·7	4·2
	6·7	*5·0*	*7·0*	*7·0*	*8·5*	*7·5*	*7·0*	*4·5*	*8·5*	*8·0*
	5·1	2·1	1·3	4·2	8·4	4·6	2·6	2·5	5·0	5·2
	7·0	*7·2*	*6·0*	*8·5*	*11·5*	*6·0*	*6·0*	*5·0*	*6·7*	*9·5*
	5·6	2·2	2·2	5·1	8·7	3·8	4·6	4·7	3·3	6·1
	9·2	*7·5*	*7·0*	*9·0*	*10·0*	*7·0*	*9·0*	*11·0*	*6·5*	*11·0*
	4·5	5·6	7·6	8·1	6·8	6·6	6·3	7·5	6·2	7·6
	6·0	*8·5*	*10·2*	*11·5*	*10·0*	*10·£*	*10·0*	*12·2*	*12·0*	*8·0*
Total ...	80·4	45·4	36·2	57·8	94·8	66·3	47·2	51·4	57·4	68·3
	100·8	*89·4*	*79·3*	*96·0*	*122·5*	*111·2*	*97·0*	*108·5*	*103·8*	*117·8*
Mean ...	5·74	3·24	2·59	4·13	6·77	4·74	3·37	3·67	4·10	4·88
	7·20	*6·39*	*5·66*	*6·86*	*8·75*	*7·94*	*6·93*	*7·75*	*7·41*	*8·41*

		Field 14					Field 26		
7·5	7·7	8·6	7·6	6·2	7·8	9·3	9·7	8·8	7·5
9·7	*10·2*	*12·0*	*15·2*	*9·2*	*12·5*	*13·7*	*13·5*	*11·5*	*9·0*
6·5	6·1	5·7	5·5	6·9	9·1	7·2	6·6	6·9	7·6
6·5	*6·2*	*6·0*	*9·0*	*8·2*	*11·0*	*9·5*	*8·5*	*7·5*	*8·5*
6·9	6·3	4·7	6·3	7·5	9·0	7·1	6·2	6·7	7·8
8·7	*8·0*	*6·0*	*8·5*	*12·0*	*11·5*	*9·0*	*8·0*	*7·5*	*10·5*
7·4	6·9	5·5	7·0	7·2	9·2	7·0	6·3	5·8	6·6
8·5	*8·5*	*6·5*	*8·0*	*11·5*	*11·2*	*9·5*	*8·0*	*6·5*	*7·7*
6·6	7·0	5·9	7·1	6·9	8·4	5·8	5·7	4·2	4·0
8·5	*8·2*	*6·2*	*10·0*	*10·5*	*10·2*	*8·2*	*7·0*	*5·2*	*6·0*
9·1	6·9	5·8	7·3	6·8	8·8	6·3	4·7	3·9	4·2
10·0	*9·5*	*8·5*	*12·2*	*12·5*	*11·0*	*7·5*	*6·5*	*6·0*	*5·7*
7·2	7·5	5·7	7·2	7·2	7·8	6·0	4·5	3·7	4·7
9·5	*9·2*	*6·5*	*8·0*	*10·0*	*8·5*	*7·0*	*6·5*	*5·7*	*6·0*
7·0	7·3	5·9	7·4	7·9	8·1	5·0	4·6	4·6	6·4
8·2	*9·5*	*6·5*	*8·7*	*12·5*	*11·5*	*6·5*	*6·2*	*5·5*	*10·5*
7·4	6·8	5·0	6·7	8·0	6·6	3·9	3·5	4·6	5·4
8·0	*6·5*	*6·2*	*8·2*	*11·0*	*8·5*	*4·5*	*4·5*	*6·2*	*6·5*
7·8	6·9	6·1	7·7	6·7	6·1	4·1	4·0	4·9	5·6
11·2	*10·2*	*7·2*	*10·0*	*11·5*	*8·2*	*5·0*	*5·5*	*6·0*	*6·5*
8·1	7·4	5·2	6·6	7·8	6·8	5·5	5·6	5·4	6·3
12·0	*9·5*	*7·7*	*11·5*	*13·7*	*8·0*	*6·5*	*6·5*	*7·0*	*7·5*
7·9	6·0	6·1	6·5	7·2	7·4	6·6	6·1	6·6	5·9
10·5	*8·5*	*8·5*	*10·5*	*12·0*	*10·0*	*7·5*	*8·5*	*7·5*	*8·2*
7·9	6·9	7·5	7·6	7·1	7·4	5·9	6·0	6·2	7·4
10·0	*7·0*	*9·5*	*11·0*	*12·0*	*8·5*	*6·2*	*6·5*	*6·5*	*8·0*
8·1	8·2	8·1	7·8	8·5	7·0	7·2	8·0	4·5	7·2
10·5	*13·0*	*13·0*	*13·5*	*13·0*	*9·0*	*8·5*	*10·2*	*7·5*	*8·5*
Total ... 105·4	97·9	85·8	98·3	101·9	109·5	86·9	81·5	76·8	86·6
131·8	*124·0*	*110·3*	*144·3*	*159·6*	*139·6*	*109·1*	*105·9*	*96·1*	*109·1*
Mean ... 7·53	6·99	6·13	7·02	7·28	7·82	6·21	5·82	5·49	6·19
9·41	*8·86*	*7·88*	*10·31*	*11·40*	*9·97*	*7·71*	*7·56*	*6·86*	*7·79*

		Field 27					Field 28		
5·8	6·0	6·7	8·0	6·6	8·6	7·6	7·7	7·7	8·6
8·5	*12·7*	*14·7*	*10·0*	*11·5*	*13·0*	*13·0*	*12·0*	*12·0*	*11·7*
5·2	5·8	5·4	5·8	6·6	8·7	7·6	6·7	8·2	8·7
10·2	*8·7*	*10·7*	*8·2*	*8·5*	*10·7*	*8·5*	*8·0*	*9·5*	*10·2*
6·6	7·4	7·4	5·2	5·6	8·1	7·7	5·8	7·1	8·2
8·7	*9·0*	*9·5*	*8·5*	*11·0*	*11·5*	*10·2*	*8·7*	*12·0*	*11·2*
7·7	6·1	7·0	6·2	7·8	8·7	8·0	6·6	7·6	8·1
9·2	*8·7*	*9·5*	*8·2*	*9·5*	*10·7*	*10·7*	*7·5*	*11·5*	*10·7*
7·4	6·8	7·2	6·6	8·2	8·8	8·6	6·1	8·7	7·7
10·0	*10·0*	*11·5*	*8·2*	*10·7*	*12·5*	*12·0*	*8·0*	*13·0*	*12·5*
7·0	5·9	6·0	7·1	7·6	8·9	8·1	7·1	8·5	8·2
10·0	*9·5*	*9·0*	*10·5*	*11·2*	*12·2*	*12·0*	*9·0*	*12·0*	*11·0*
6·7	6·9	6·1	6·0	7·3	8·7	8·8	5·9	8·2	8·5
8·5	*8·7*	*6·5*	*7·2*	*9·2*	*11·0*	*11·0*	*8·0*	*11·0*	*10·5*
6·2	6·3	5·2	6·7	9·2	9·1	7·8	5·6	6·6	8·6
9·0	*8·5*	*6·5*	*8·0*	*11·5*	*12·0*	*11·0*	*7·5*	*8·7*	*11·0*
6·5	6·4	6·2	6·7	8·8	9·2	8·6	5·1	6·7	8·6
8·2	*9·0*	*8·0*	*10·0*	*11·5*	*11·2*	*12·0*	*7·0*	*8·5*	*12·0*
7·5	6·2	5·6	5·8	7·4	8·7	7·9	5·5	7·1	8·0
10·0	*8·2*	*7·0*	*8·0*	*9·0*	*12·0*	*9·0*	*6·0*	*11·5*	*8·5*
7·7	6·7	5·4	5·4	6·7	8·3	7·3	5·0	7·3	7·0
9·7	*8·7*	*7·5*	*8·0*	*9·0*	*12·0*	*9·5*	*6·5*	*10·0*	*9·5*
7·5	5·6	4·7	6·7	5·8	8·7	7·1	5·0	7·1	7·5
11·2	*8·2*	*8·5*	*8·0*	*9·5*	*12·0*	*10·0*	*6·0*	*11·0*	*9·0*
6·1	4·1	4·3	4·6	5·2	8·0	5·7	5·3	6·7	7·2
7·0	*5·5*	*6·0*	*6·5*	*8·0*	*11·5*	*8·0*	*8·0*	*11·5*	*9·0*
7·2	7·2	6·0	5·8	5·9	7·9	7·8	8·0	6·4	7·0
8·0	*9·0*	*8·0*	*7·0*	*7·5*	*11·0*	*11·0*	*11·0*	*7·0*	*8·0*
Total ... 95·1	87·4	83·2	86·6	98·7	120·4	108·6	85·4	103·9	111·9
128·2	*124·4*	*122·9*	*116·3*	*137·6*	*163·3*	*147·9*	*113·2*	*149·2*	*144·8*
Mean ... 6·79	6·24	5·94	6·19	7·05	8·60	7·76	6·10	7·42	7·99
9·16	*8·89*	*8·78*	*8·31*	*9·83*	*11·66*	*10·56*	*8·09*	*10·66*	*10·34*

NOTE ON A METHOD FOR THE PREPARATION OF PERMANENT RECORDS OF SOIL COLOUR.

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HART[1] has recently emphasised the importance of obtaining soil colour data. The following method, which is an elaboration of one proposed by Monie[2], has proved satisfactory in practice.

Apparatus required.

Enamel cups or evaporating basins, rubber pestle, fountain pen filler, Gooch crucibles, wash bottle, hard filter paper discs to fit crucibles, filter flask and pump, celluloid varnish (bronze medium 492), soft camel-hair brush, vegetable glue.

Manipulations.

A filter paper disc is marked with the sample number and placed in the crucible, the numbered surface downwards, the crucible placed in position in the filter flask and the pump started. A small quantity (about 5 gm.) of the air dry soil, which has been passed through a 2 mm. sieve, is placed in an enamel cup or evaporating basin. Water (about 20–25 c.c.) is added and the sample triturated with a rubber pestle so as to form a suspension. The filter paper in the Gooch crucible is moistened with water from a wash bottle and, when sucked dry by the pump, a few drops of the freshly stirred suspension are allowed to fall on the filter disc by means of the fountain pen filler, allowing each drop to be sucked dry before adding another. This process is continued till the disc is coated with a thin film of soil and the white filter paper is no longer visible.

After drying the Gooch crucible and its contents in the steam oven, the disc is readily removed from the crucible by a sharp blow against the palm of the hand. The disc is then coated with celluloid varnish by means of a camel-hair brush, care being taken that the bristles of the brush do not touch the soil film. The disc is then allowed to dry overnight in the air without heating, as this causes curling. When dry the disc can be handled freely as the celluloid varnish cements the soil particles firmly to the paper.

For convenience, the discs are mounted on index cards on which the analytical data are recorded. It has been found that a small sheet of aluminium having a circular hole of the same diameter as the paper disc is of great assistance in mounting. This shield is placed on the index card and the area of the card exposed by the hole smeared with vegetable glue. The shield is removed and the appropriate disc placed in position on the card and pressed firmly into position, using blotting paper to absorb the surplus glue. The cards are finally pressed between layers of blotting paper in a screw press.

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THE INFLUENCE OF SYSTEMATIC PLOT ARRANGEMENT UPON THE ESTIMATE OF ERROR IN FIELD EXPERIMENTS.

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(With Four Text-figures.)

INTRODUCTION.

ONE of the most valuable methods of plot arrangement is that in which the number of replicates is equal to the number of treatments or varieties, plots being arranged in a square, so that each treatment appears once in each row and once in each column. By this method it is possible to subtract that part which is due to differences between columns or rows from the total variation in a field experiment. Scandinavian authors, Kristensen(3, 4), Lindhard(5), Vik(6), and others in other countries have devised systems for laying out the plots in this way. The object chiefly held in view, besides the elimination of the "systematic" variation in the field, was that different replicates of the same treatment should be removed as far from one another as possible, in order to minimise the soil differences between the different treatments. As the best schemes of arrangement for the 5 × 5 square, the "knight's move" has been devised, two variants of which are represented in arrangements 1 and 2 in Fig. 1 of the present paper. In contrast, the "diagonal" arrangements, as shown in 3 and 4 of the same figure, are considered very poor.

In attempting to reconcile the advantages of the elimination of soil error with the theoretical requirement of a valid estimate of error, Fisher was early attracted to arrangements of this type(1). He showed that of the numerous possible arrangements subject to the condition that each treatment should appear once in each row and once in each column, the enumeration of which had been considered by Euler as the problem of the Latin Square, it was possible to choose at random one to be used in the field, and that when this was done the estimate of error would certainly be statistically valid. Arrangements arrived at in this way he therefore called Latin Square arrangements, in contradistinction to systematic forms of arrangement in a square, the use of which would lead to an incorrect estimate of error.

If a large number of trials has to be made each year, the use in different trials of different arrangements will considerably increase labour, risks of mistakes in sowing, manuring, and so on. It therefore seems to be of interest to know to what extent the regular use of a systematic arrangement will influence the estimate of the error. This question is not purely mathematical, and it cannot be solved by deductive methods only. However, by testing the arrangements in question on a considerable number of blocks of uniformity trials, valuable information may be obtained about their influence upon the estimate of error. Such an empirical study is the main issue of the present investigation.

The author wishes to express his most cordial thanks to Dr R. A. Fisher of the Rothamsted Experimental Station. The idea of this study was conceived during a lecture by Dr Fisher at a meeting in Copenhagen of the field-plot-technique section of the Scandinavian Association of Agricultural Science (Nordisk Jordbruksforskningsförening). Personally, after the meeting, and then in several letters, Dr Fisher has most kindly offered very valuable advice and information which has enabled the author to carry the investigation to its completion.

MATERIAL AND METHODS.

The following methods have been employed in this investigation: In blocks of 5×5 plots, all treated in the same manner and carrying the same variety of crop, the total variance of the block is determined, and then the variation in columns and rows is eliminated by the methods given by Fisher[2]. Then five "treatments" are distributed according to different plans, maps of which may be found in Fig. 1 of this paper. Arrangements 1 and 2 are knight's moves, 3 and 4 are diagonals. Arrangement 12 is constructed for a special purpose to be discussed later on, and arrangements 5 to 11, finally, are random arrangements.

```
3 4 5 1 2     2 1 5 4 3     2 3 4 5 1     1 5 4 3 2     3 1 2 4 5     4 3 1 5 2
5 1 2 3 4     4 3 2 1 5     3 4 5 1 2     2 1 5 4 3     4 5 1 3 2     1 5 2 3 4
2 3 4 5 1     1 5 4 3 2     4 5 1 2 3     3 2 1 5 4     2 4 3 5 1     5 2 4 1 3
4 5 1 2 3     3 2 1 5 4     5 1 2 3 4     4 3 2 1 5     1 3 5 2 4     2 1 3 4 5
1 2 3 4 5     5 4 3 2 1     1 2 3 4 5     5 4 3 2 1     5 2 4 1 3     3 4 5 2 1
    Arr. 1        Arr. 2        Arr. 3        Arr. 4        Arr. 5        Arr. 6

5 4 1 2 3     3 2 1 4 5     4 3 1 5 2     1 2 4 5 3     5 3 1 2 4     4 5 2 3 1
4 2 3 1 5     5 4 3 1 2     5 1 3 2 4     2 5 3 1 4     1 4 3 5 2     5 2 3 1 4
3 1 4 5 2     4 1 2 5 3     1 5 2 4 3     5 3 2 4 1     2 1 5 4 3     2 4 1 5 3
2 3 5 4 1     1 3 5 2 4     2 4 5 3 1     3 4 1 2 5     3 2 4 1 5     3 1 4 2 5
1 5 2 3 4     2 5 4 3 1     3 2 4 1 5     4 1 5 3 2     4 5 2 3 1     1 3 5 4 2
    Arr. 7        Arr. 8        Arr. 9       Arr. 10       Arr. 11       Arr. 12
```

Fig. 1. Plans of the different arrangements used.

Of the sixteen degrees of freedom which remain after the elimination of those of columns and rows, four may thus be considered as belonging to "differences of treatment." The $S(d^2)$ corresponding to these four degrees of freedom is determined as a percentage of the $S(d^2)$ of all sixteen degrees of freedom, and the values thus obtained are called "treatment-error-coefficients" of the special arrangements and blocks, abbreviated t.e.c.

The material is taken from uniformity trials, available in the literature, and a list of the trials used is given in Table I, together with data on plot size and on crop.

Table I.

Field	Quotation from	Size of Plots	Crop	Cases
I (Askov)	R. K. Kristensen in Tidsskrift for Planteavl (1925), **31**, 467	6·2 × 8·8 m.	Barley	1–8
II (Mercer and Hall)	Harris in American Naturalist (1915), **49**, 435	$\frac{1}{200}$ acre	Mangolds; only the data for roots are used	9–16
III (Mercer and Hall)	Harris in American Naturalist (1915), **49**, 436–7	$\frac{1}{500}$ acre	Wheat; only the data for grain are used	17–36
IV (Montgomery)	Harris in American Naturalist (1915), **49**, 440	—	Wheat; only the data on weight of grain are used	37–42
V	B. R. Larsen in Tidsskrift for Planteavl (1906), **12**, 347	5 × 5 m.	Timothy hay	43–50
VI (Scofield)	Harris in J. Agric. Res. (1920), **19**, 279–314	23¼ × 79¼ ft.	Alfalfa, second cutting 1913; first and second cuttings 1914; ear corn 1915 and 1916	51–70
VI a (Same as VI)	Harris in J. Agric. Res. (1920), **19**, 279–314	23¼ × 105⅝ ft.	Alfalfa, third cutting 1914	71–74
VII	N. A. Hansen in Tidsskrift for Planteavl (1915), **21**, 553, "Mark A 2"	25 used somewhat more than 50 sq. m.	Different crops in five different years 1907, oats; 1908, rye; 1909, barley; 1910, swedes; 1911, barley	75–79
VIII	N. A. Hansen in Tidsskrift for Planteavl (1915), **21**, 553, "Mark E 2"	Somewhat more than 50 sq. m.	Different crops in four different years 1906, oats; 1907, barley; 1908, hay; 1909, rye	80–91

For all, sixty-two blocks of 5 × 5 plots have been tested, but some of these blocks have carried several different crops, and the total number of different cases tested amounts to ninety-one. More material would have been desirable, but, unfortunately, no more has been available, and the material studied throws considerable light on the problem.

TREATMENT-ERROR-COEFFICIENTS OF DIFFERENT ARRANGEMENTS.

The main bulk of the results obtained is presented in Table II. As a detailed publication of the 1092 t.e.c.'s, together with the variance of the ninety-one blocks, would require too much space, the data have been condensed in the manner seen in the table. The meaning of the values x_1 and x_2 will be explained later.

Table II. *Distribution of treatment-error-coefficients of arrangements 1–12.*

Arrangement... t.e.c.	1	2	3	4	5	6	7	8	9	10	11	12	Total
0	4	3	4	6	3	6	7	1	6	4	3	1	48
5	21	14	7	6	7	13	11	4	9	12	15	15	134
10	8	9	15	7	21	13	5	12	5	10	16	7	128
15	12	23	7	17	13	12	15	19	10	16	13	12	169
20	9	10	12	11	9	13	15	10	16	12	10	9	136
25	9	9	13	5	9	18	10	12	17	14	12	16	144
30	9	7	8	13	8	5	9	6	9	8	8	12	102
35	8	4	10	4	2	4	6	9	5	6	4	4	66
40	4	6	5	4	7	4	5	6	6	1	—	5	53
45	3	1	4	9	4	2	5	3	4	6	2	2	45
50	1	1	4	2	2	1	1	6	1	1	3	5	28
55	1	3	1	4	3	—	1	2	3	—	—	3	21
60	1	—	—	1	1	—	1	—	—	1	2	—	7
65	1	—	—	1	2	—	—	1	—	—	1	—	6
70	—	1	—	—	—	—	—	—	—	—	1	—	2
75	—	—	—	1	—	—	—	—	—	—	1	—	2
80	—	—	1	—	—	—	—	—	—	—	—	—	1
85													
Total ...	91	91	91	91	91	91	91	91	91	91	91	91	1092
Mean ...	22·77	22·83	26·24	27·94	25·25	20·91	23·93	27·45	25·25	22·94	23·16	25·69	24·53
x_1 ...	44·72	44·72	37·07	37·07	42·98	42·39	39·52	42·49	38·67	38·61	40·70	36·41	
x_2 ...	0	0	16	16	5	5	8	6	12	10	8	13	

After the elimination of columns and rows there remain sixteen degrees of freedom, and since four of these correspond to treatments, the mean value of t.e.c. should be 25. For all twelve arrangements the mean t.e.c. is 24·53, for the random arrangements 5–11 it is only 24·13, a somewhat large deviation from expectation. However, as the number

of arrangements tested is very small in comparison with the number possible, this deviation has no real significance.

It was presumed that the knight's move arrangements would give the smallest possible differences between "treatments," and, in fact, the mean t.e.c.'s of the two knight's moves are 22·72 and 22·83 respectively.

On the other hand, the diagonal arrangements were expected to give high t.e.c. values, and they have given 26·24 and 27·94 respectively.

The deviations of the mean t.e.c.'s of the systematic arrangements from 25 are rather small, and perhaps it is somewhat doubtful whether they are fully significant. As the distribution of the individual values is far from normal, the variance, standard error, and standard error of the mean do not give complete information about the significance. It may be of interest, however, to know these values, and they have therefore been calculated. The standard errors of the means of the four systematic arrangements are:

Arrangement 1	...	...	± 1·554
,, 2	...	...	± 1·498
,, 3	...	...	± 1·557
,, 4	...	...	± 1·746

There seems to be no logical reason why the two knight's moves, *inter se*, or the two diagonals, should be really different, and as the above figures do not indicate such differences it is considered justifiable to combine the values of arrangements 1 and 2 as well as those of arrangements 3 and 4. For the knight's move we then obtain an average t.e.c. of 22·80 ± 1·076; for the diagonal the corresponding value is 27·09 ± 1·169. The deviations from 25 are about twice the standard error, and are thus rather significant but not fully conclusive. On the other hand, it seems fairly safe to conclude that the true mean value of the knight's move is not less than 20, while that of the diagonal is not more than 30.

It may be of interest to see how much a deviation in the mean t.e.c. will, on an average, influence the estimate of error and that of the apparent significance of differences between treatments. If the t.e.c. is only 20, the estimate of variance, obtained from the twelve degrees of freedom within treatments, will stand to the true variance between treatments as 80/12 : 20/4, or as 1·333 : 1. Then the estimate of standard error will stand to the true standard error as 1·155 : 1. In estimating the significance of a difference by means of $t_{(2)}$, the true value of t will stand to the estimated as 1·155 : 1. With $n = 12$, P is 0·05 when t is

2·179. An observed t of 2·179 corresponds to a true t of 2·517, which gives a P of only 0·030. The relation between estimated and true variance, between estimated and true standard error, and the percentage of cases exceeding the 0·05 point for the t.e.c. values 20–30 inclusive, are given in Table III.

<div align="center">

Table III.

</div>

t.e.c.	Estimated variance True variance	Estimated error True error	% of cases above the 0·05 point
20	1·333 : 1	1·155 : 1	3·0
21	1·254 : 1	1·120 : 1	3·4
22	1·182 : 1	1·087 : 1	3·9
23	1·116 : 1	1·056 : 1	4·3
24	1·056 : 1	1·028 : 1	4·7
25	1 : 1	1 : 1	5·0
26	0·949 : 1	0·974 : 1	5·7
27	0·901 : 1	0·949 : 1	6·4
28	0·857 : 1	0·926 : 1	7·0
29	0·816 : 1	0·904 : 1	7·6
30	0·778 : 1	0·882 : 1	8·3

It is evident that the use of arrangements with a mean t.e.c. differing by a few units from 25 will cause considerable faults in the estimate of error and in judging the significance of differences.

In the study of the influence of systematic plot arrangements upon the t.e.c. evidently not only the mean t.e.c. value has to be considered but also the distribution of the individual values. Dr Fisher has kindly supplied a brief table of the random distribution of the t.e.c. values, giving the limits within which 10, 20, 30 per cent. of all t.e.c. values should fall. These limits are found in the column heads of Table IV. Thus 10 per cent. of the values should fall below 7·88, 10 per cent. should fall between this value and 11·95, another 10 per cent. between 11·95 and 15·59 and so on.

<div align="center">

Table IV. *Distribution of the t.e.c. values with different plot arrangements.*

</div>

Arrangement	Number of t.e.c. values between									Expected number in each class	χ^2	P
	0 – 7·88	7·88 – 11·95	11·95 – 15·59	15·59 – 19·16	19·16 – 22·85	22·85 – 26·85	26·85 – 31·43	31·43 – 37·09	37·09 – 45·26	45·26 – 100			
1	16	12	7	10	7	2	11	12	7	7	9·1	15·044	0·09
2	7	13	7	20	9	6	9	4	10	6	9·1	19·483	0·02
1–2	23	25	14	30	16	8	20	16	17	13	18·2	20·418	0·016
3	10	7	10	4	12	8	9	10	11	10	9·1	5·154	0·82
4	9	5	6	15	8	6	6	13	5	18	9·1	20·051	0·018
3–4	19	12	16	19	20	14	15	23	16	28	18·2	10·967	0·28

Tests of homogeneity: 1 and 2: $\chi^2 = 13.951$; $P = 0.13$.
3 and 4: $\chi^2 = 14.367$; $P = 0.11$.
1 + 2 and 3 + 4: $\chi^2 = 18.080$; $P = 0.04$.

The table presents the actual distributions for the four systematic arrangements and also the combined values of the two knight's moves, as well as those of the two diagonals. The expected number in each class, the value of χ^2 and the corresponding value of P ($n = 9$) are also shown. Tests of homogeneity have been made between arrangements 1 and 2, and between arrangements 3 and 4, and the values of χ^2 and P are given below the table. The values of P being only 0·13 and 0·11 respectively there is some indication that in the material studied arrangements 1 and 2 and arrangements 3 and 4 are not absolutely equivalent. As discussed later on there must be a negative correlation between the t.e.c. values of the arrangements of the same type, and in a comparatively small number of cases this correlation may cause some amount of difference between the t.e.c. distribution of the two arrangements. On the other hand, the correlation mentioned is rather slight, and in a large material there is no reason why the one diagonal should be essentially different from the other, or the one knight's move from the other. Thus there does not seem to be any cogent objection against the combining of the values from arrangements of the same type.

The distributions of the knight's move arrangements are significantly different from the expected distribution, but at first sight the deviations seem to be rather irregular. Of the two diagonals, number 3 shows a surprisingly good fit with the random distribution, but number 4 deviates significantly; the combined values, however, do not show a significant deviation.

As stated above, the deviations from the expected distribution seem to be quite irregular. It is possible, however, by means of the methods presented ((2), chap. v) to distinguish between deviations ascribable to a linear regression and the remaining deviations. If $a_1 \ldots a_{10}$ represent the frequencies of t.e.c. in the different classes, which by random arrangements should include each 10 per cent. of the values, then the function

$$4\tfrac{1}{2}\,(a_{10} - a_1) + 3\tfrac{1}{2}\,(a_9 - a_2) + 2\tfrac{1}{2}\,(a_8 - a_3) + 1\tfrac{1}{2}\,(a_7 - a_4) + \tfrac{1}{2}\,(a_6 - a_5)$$

has the value -87 and $+63$ for the knight's moves and the diagonals respectively. The standard deviation of these values ((2), p. 246) is 38·6. Thus $(-87/38·6)^2 = 5·081$, and $(63/38·6)^2 = 2·663$, are the values of χ^2 corresponding to the linear regressions of a upon the class number. Since this regression includes only one degree of freedom, it is significant in the case of the knight's move, but insignificant in the case of the diagonal. (The individual class frequencies and the linear regression lines are plotted in Figs. 2 and 3.) Thus even by this method it has not been
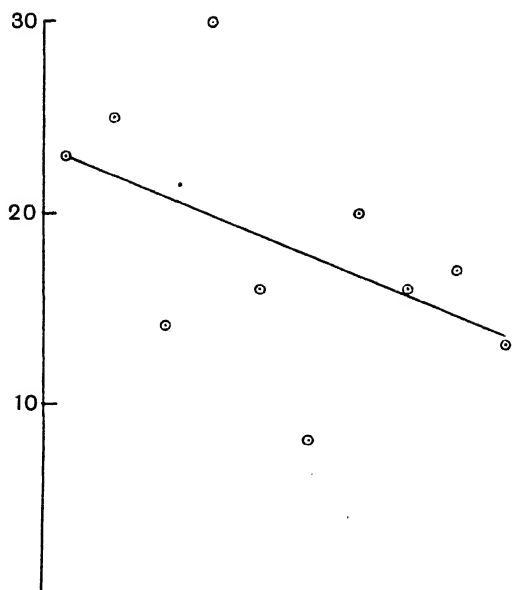
Fig. 2. Knight's move. Chart of the class-frequencies of Table IV, together with the straight regression line.
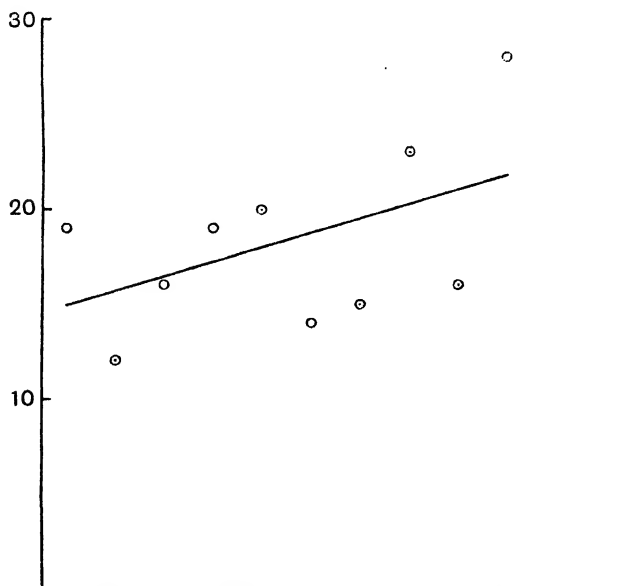
Fig. 3. Diagonal. Chart of the class-frequencies of Table IV, together with the straight regression line.

possible to demonstrate that the t.e.c.'s of the diagonal arrangements are distributed in essentially another way than should be expected by random arrangement, whereas the deviations of the knight's move have proved to be significant.

<div align="center">RELATION BETWEEN TREATMENT-ERROR-COEFFICIENT
AND SPREADING OF PLOTS.</div>

The elimination of the variation between columns and rows in the 5×5 block may be carried out in a somewhat different manner than the one presented by Fisher[2], involving somewhat heavier arithmetic, but offering for some purposes a more clear view of what has been done. This method has been used, among others, by Kristensen[4], and includes the "correction" of the yield of the individual plot in such a way that all columns and all rows will have the same yield, whereas the total yield and the averages of the different treatments remain unaltered. This correction being made, the $S(d^2)$ of the corrected values will be equal to the remaining $S(d^2)$ after elimination of the parts belonging to columns and rows by the method of Fisher.

Now, if the "corrected" plot values were distributed completely at random, then any systematic arrangement, regularly used, could not change this "natural randomisation," but the t.e.c.'s of any arrangement would give the theoretical random distribution. The strong indications that the t.e.c.'s of systematic arrangements are distributed in another manner must therefore indicate that the "corrected" plot values are not distributed wholly at random, but governed to some extent by one rule or the other. Most likely this rule is a certain degree of correlation between adjacent plots. If such a correlation exists, then arrangements where adjacent plots often have the same treatment should give a high value of t.e.c., and arrangements where adjacent plots never have the same treatment should give low values. It should then be possible to characterise, in one way or the other, the degree of "spreading" of the plots with the same treatment, and thus to obtain a "spreading coefficient" of the different arrangements, which should show a high degree of correlation with the mean t.e.c. values of the arrangements.

Several such "coefficients of spreading" have been tested, but only two of them seem to be of any value. One of them is calculated in the following way. The plots are presumed to be squares with unit side, and the centres of the five plots of one treatment are combined with the four shortest possible lines. (Fig. 4 shows the method with one treatment.) The total length of the 5×4 lines is then used as the "spreading

coefficient" of the arrangement. The spreading coefficients of the twelve arrangements in this paper are presented in Table II as x_1. As no single treatment can have a higher coefficient than $4\sqrt{5}$, the knight's move, where all the treatments have just this coefficient, must represent the highest degree of spreading, according to this method of determination. The diagonal, however, does not represent the opposite extreme, but arrangements may be obtained with a still lower coefficient. Arrangement 12 in this paper represents such an arrangement, constructed for this special purpose.
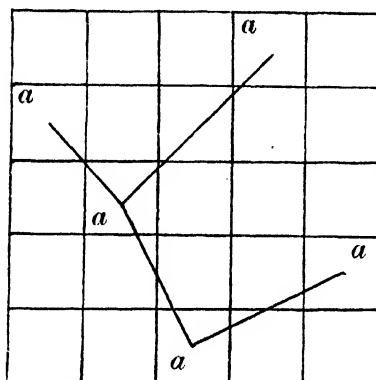
Fig. 4. Method for the calculation of the "spreading-coefficient." Coefficient of treatment $a = \sqrt{2} + 2\sqrt{5} + \sqrt{8} = 8.71$.

If y is the mean t.e.c. of the arrangement, then the regression coefficient, y upon x_1, is -0.351. The significance of this regression is tested by means of the analysis of variance and the use of z, as presented by Fisher ((2), chaps. vii–viii). The results of the analysis of variance are tabulated in Table V.

Table V.

	D.F.	$S(d^2)$	Mean square
Total	1091	228,782	—
Intraclass	1080	224,294	207.7
Interclass	11	4,488	408.0
Regression	1	1,089	1089.0
Remaining interclass	10	3,399	339.9

For the difference regression—intraclass $z = 0.8285$, beyond the 0.05 point, and the regression is significant. The (remaining interclass)—(intraclass) difference has $z = 0.2463$ and is thus insignificant. However, one-third of the $S(d^2)$ of remaining interclass is contributed by arrangement 8, another one-third by arrangements 6 and 10, and it

does not seem safe to conclude that the regression accounts for all the differences between the t.e.c.'s of different arrangements.

The "coefficient of spreading" takes into account not only the instances where two neighbouring plots have the same treatment, but also such where one plot of the five is separated from the other by a comparatively long distance. Another index may be constructed which only accounts for the cases of close neighbourhood between equally treated plots. It is possible in sixteen different ways to take a block of 2×2 plots from a 5×5 block. As soon as two plots in such a 2×2 block have the same treatment, the arrangement is given one point; and if there are only two arrangements within one block. this evidently means two points. The values of this index are given in Table II as x_2. Even in respect to this index the knight's move represents one extreme, as $x_2 = 0$ for arrangements 1 and 2. And here the diagonal represents the other extreme, having $x_2 = 16$. The only case where this number may be exceeded is when one block of 2×2 has two points. Then this block must be of the type $\begin{vmatrix} a & b \\ b & a \end{vmatrix}$. As none of the neighbouring plots can be a or b, the next 2×2 block will have no points, and one point per block, or sixteen in all, is the maximum.

The regression coefficient of mean t.e.c. upon x_2 is $+ 0 \cdot 239$, and the analysis of variance gives the following result as shown in Table VI:

Table VI.

	D.F.	$S(d^2)$	Mean square
Total	1091	228,782	—
Intraclass	1080	224,294	207·7
Interclass	11	4,488	408·0
Regression	1	1,674	1674·0
Remaining interclass	10	2,814	281·4

The difference regression—intraclass has $z = 1 \cdot 063$, which is beyond the $0 \cdot 01$ point, and the regression is to be considered as very significant. The remaining interclass is quite insignificantly larger than the intraclass. Still, arrangements 6 and 8 contribute the major part of the deviations from the regression line, and it does not seem safe to maintain that the mean t.e.c. of an arrangement is determined only by the value of the index x_2.

Anyway, the existence of a clearly significant regression between this index and the t.e.c. of the arrangement has not only confirmed the conclusions of the previous section, i.e. that the t.e.c. of the knight's move differs from random expectation, but it also shows that the true t.e.c.

of the diagonal arrangement must deviate about equally much from random expectation.

Further, it must be possible, by determining the index x_2 of any given arrangement, to predict with a fairly high probability whether this arrangement will have a low or a high t.e.c., or a medium value.

It would be of great help to the practical field-plot experimenter if an arrangement existed that would give a distribution of t.e.c. closely approximating the random distribution, but to conclude from the above results that any arrangement with x_2 about 8 would fulfil this requirement is evidently unwarranted.

RELATIONS BETWEEN TREATMENT-ERROR-COEFFICIENT AND THE CONDITIONS OF THE EXPERIMENT.

In the previous discussion the material from all the different fields has been pooled, and in spite of the fact that the fields differ widely in regard to plot size, plot shape, crop, soil and climatic conditions, the results have been used for general conclusions. In order to test the reliability of this generalisation it is necessary to know whether there exist any differences between the different fields, or if there is any relation between the values of t.e.c. and certain conditions of the experiment.

There are nine fields, VI and VI *a* being counted as different fields, and as the variance of t.e.c. is very great there are evidently great differences between the mean t.e.c.'s of one arrangement on different fields. The significance of these differences may be tested by the method given by Fisher ((2), chaps. vii and viii), *i.e.* by a comparison of the interclass with the intraclass variance, when the field is considered as class. For arrangement 1 we obtain the analysis of variance given in Table VII.

Table VII.

	D.F.	$S(d^2)$	Mean square
Total	90	19,772	219·7
Within fields	82	17,747	216·4
Between fields	8	2,025	253·2

The value of z is only 0·078, and there is no indication that arrangement 1 should have essentially different effects in different fields.

The same analysis is carried through with all twelve arrangements, and in Table VIII the z values obtained are presented:

Table VIII.

Arrangement	z	Arrangement	z	Arrangement	z
1	0·0784	5	0·1281	9	−0·1019
2	−0·1091	6	0·4088	10	0·0942
3	0·2314	7	−0·3887	11	−0·3595
4	0·0813	8	−0·5371	12	0·4023

Arrangements 2, 7, 8, 9 and 11 have smaller variances between fields than within fields; in the remaining seven arrangements the interclass variance is the largest, and in two cases, arrangements 6 and 12, z is between the 0·05 and the 0·01 points. Perhaps the results indicate that in some instances differences between experimental conditions may essentially change the t.e.c. of a certain arrangement, but on the whole they do not invalidate the general conclusions of the previous sections.

There is no reason to believe that the variability of the 5 × 5 block as a whole should have any influence upon the value of t.e.c., but, nevertheless, the existence of a relation between variability and t.e.c. has been looked for in arrangements 1, 2 and 3. The percentage standard deviation of each block of 5 × 5 has been determined, and the variance of these values has been analysed with the material grouped in classes according to the value of t.e.c. of arrangements 1, 2 and 3 respectively. The total percentage standard deviation, as well as the percentage standard deviation after elimination of the part belonging to columns and rows, has been studied. Not only has the interclass variance been contrasted with the intraclass, but the eventual linear regression of percentage standard deviation upon t.e.c. has also been determined. In no single instance is there a significant interclass correlation or a significant regression. Although the study includes only three arrangements, it seems safe to conclude that the t.e.c. of any arrangement in a certain block is completely independent of the variability of that block.

It was stated in a previous section that even after the correction for columns and rows there is most probably a certain degree of correlation between the yield of adjacent plots. It would not seem quite improbable that the degree of this correlation is in some way or other related to the degree of correlation within columns and rows. If such a relation exists, there must also be a correlation between the t.e.c. value of the systematic arrangement and the strength of correlation within columns and rows. This latter correlation might have been determined in the usual way as an intraclass correlation, but the relation between the total standard deviation of the block and the standard deviation after elimination of columns and rows also gives a fair measure of the same correlation.

Thus the reduced standard deviation in percentage of the total standard deviation has been taken as an index of the correlation within columns and rows, an index that decreases with increasing correlation. Then the variance of this index with the material grouped in classes according to the values of t.e.c. has been analysed. Table IX presents the essential data of this study.

Table IX. *Relation between t.e.c.'s of different plans and the degree of correlation within columns and rows.*

Number of cases (n) with t.e.c. as indicated in column 1, and the mean value (M) for these cases of the index $100 \dfrac{\text{reduced s.d.}}{\text{total s.d.}}$

Values of t.e.c. between	Arrangement 1		Arrangement 2		Arrangement 3		Arrangement 4	
	n	M	n	M	n	M	n	M
0–10	25	72·16	17	69·82	11	77·73	12	56·00
10–20	20	71·00	32	68·19	22	66·18	24	71·08
20–30	18	68·72	19	70·42	25	65·88	16	72·88
30–40	17	66·47	11	67·18	18	65·33	17	74·41
40–50	7	67·43	7	59·43	9	74·11	13	63·62
50–60	2	68·00	4	78·75	5	72·00	6	66·33
60–70	2	35·50	—	—	—	—	2	81·00
70–80	—	—	1	93·00	—	—	1	74·00
80–90	—	—	—	—	1	109·00	—	—

With arrangement 1 the following analysis of variance is obtained (Table X):

Table X.

	D.F.	$S(d^2)$	Mean square
Total	90	33,095	—
Intraclass	84	30,393	361·8
Interclass	6	2,702	450·3
Regression	1	1,398	1398·0
Remaining interclass... ...	5	1,304	260·8

Since the value of z for the difference regression—intraclass is 0·6758, which is very near the 0·05 point, the regression is rather significant.

In arrangements 2, 3 and 4, however, there is no indication of a similar regression, since the values of $S(d^2)$ belonging to the regression line are only 32, 178 and 343 respectively, less than the intraclass variance.

It was shown that in arrangements 6 and 12 there were significant differences between the mean t.e.c.'s from different fields. In view of the regression between the index $100 \dfrac{\text{reduced standard deviation}}{\text{total standard deviation}}$ and the t.e.c.'s of arrangement 1, those differences might have something to do with the different mean indices of the different fields. There is no indication, however, of the existence of such a regression in arrangements

6 and 12. Thus in one case out of six tested this regression is indicated, z barely reaching the 0·05 point, to be reached by chance once in twenty cases. The conclusion seems to be warranted that the regression in arrangement 1 is a mere chance occurrence, and that the value of t.e.c. is independent of the degree of correlation within columns and rows.

Finally, the interrelation between arrangements 1 and 2, and that between 3 and 4 has been studied. Each of the "treatments" of arrangement 2 comprises one plot from each of the "treatments" of arrangement 1, and thus all that part of the variance, which appears as "treatment" in the one arrangement, will appear as "error" in the other. The same relation exists between arrangements 3 and 4. It is obvious, then, that for one and the same block the sum of the t.e.c.'s of arrangements 1 and 2 cannot exceed 100, nor the sum of the t.e.c.'s of arrangements 3 and 4. This again must lead to a certain degree of negative correlation between the t.e.c.'s of the two arrangements in one pair. Tables XI and XII give the essential data for the study of these correlations.

Table XI. *Relation between the t.e.c.'s of arrangements 1 and 2.*

t.e.c. of arrangement 1 ...	0	10	20	30	40	50	60	70
Number of cases... ...		25	20	18	17	7	2	2
Mean t.e.c. of arrangement 2		23·41	25·25	24·79	20·12	22·13	20·15	7·45

Table XII. *Relation between the t.e.c.'s of arrangements 3 and 4.*

t.e.c. of arrangement 3	0	10	20	30	40	50	60	70	80	90
Number of cases ...	11	22	25	18	9	5	—	—		1
Mean t.e.c. of arrangement 4	34·49	31·35	28·05	25·28	21·78	23·84	—	—		4·30

The analysis of variance gives the following results (Tables XIII and XIV):

Table XIII. *Arrangement 2.*

	D.F.	$S(d^2)$	Mean square
Total	90	18,369	—
Intraclass	84	17,560	209·1
Interclass	6	809	134·8
Regression	1	349	349·0
Remaining interclass	5	460	92·0

The regression is rather slight and not significant in this material, since the z value in question is only 0·2562.

Table XIV. *Arrangement* 4.

	D.F.	$S(d^2)$	Mean square
Total	90	24,977	—
Intraclass	84	23,137	275·4
Interclass	6	1,840	306·7
Regression	1	1,698	1698·0
Remaining interclass	5	142	28·4

The regression is clearly significant, since the corresponding z value is 0·9094, which is beyond the 0·05 point. The very small remaining interclass variance is striking, since z of the difference (remaining interclass)—(intraclass) is − 1·136, thus just beyond the 0·01 point. There does not seem to be any reasonable explanation of this phenomenon, and probably it is merely a chance occurrence.

The relation between the different arrangements is of no great importance to the values of t.e.c., but it has been included here since it completes the picture of the effect of systematic plot-arrangements.

Summary and conclusions.

The effect of systematic plot distribution upon the estimate of error of a field-plot experiment of 5×5 plots has been studied on ninety-one blocks taken from eight different uniformity trials, representing a wide variation in plot size and plot shape, crop, climate, and soil.

The arrangements studied are: two different knight's moves, two different diagonal arrangements, and eight irregular arrangements, seven of which have been chosen at random.

The investigation has shown that the use of systematic plans introduces a bias in the estimate of the error of the experiment. The use of the knight's move arrangement causes an over-estimation of the error, whereas the use of the diagonal causes an under-estimation.

It has further been shown that one and the same arrangement has essentially the same effect upon the estimate of error under quite different conditions. There is some indication that if individual fields are contrasted, one and the same arrangement may have different effects in the different fields, but the significance of this indication is rather slight, and the general trend of the material goes to show that the effect of an arrangement is independent of the conditions of the experiment.

It has been especially demonstrated that the degree of variability within the 5×5 block has no influence on the effect of the arrangement upon the estimate of error. This holds true with regard to the total variability as well as to the variability within columns and rows.

It has further been shown that the influence of a certain arrangement upon the estimate of error is independent of the strength of the correlation within columns and rows.

Two different indices have been calculated, by means of which it is possible to characterise an arrangement and to predict, with a fair amount of probability, whether it will lead to an over-estimation or an under-estimation of error.

It is not probable, however, that the indices mentioned completely characterise the arrangements as regards their influence upon the estimate of error. It is thus impossible at present to choose, by means of these indices, such arrangements which leave this estimate unaltered.

The result of the study gives an empirical confirmation of Fisher's theoretical considerations about the effect of using systematic plot arrangements. The theory of Fisher evidently presumes that, even since the "systematical" variation between columns and rows is eliminated, the remaining variability between plots is not distributed wholly at random. The correctness of this presumption may have been questioned, but is fully confirmed by the results of the empirical study.

Only one type of field experiment has been tested, viz. the one consisting of five treatments with five replicates each, arranged in a 5 × 5 block, each treatment occurring once in each column and once in each row. Obviously, it is dangerous to draw conclusions from this single type of experiment as to others planned in a different manner. It must be remembered, however, that even since the "systematical" part of the variation between plots is eliminated by grouping them in suitable blocks, the remaining variability is not distributed wholly at random, but is to some extent regular. This fact, proved in the study of 5 × 5 blocks, must be independent of the type of the experiment and must exercise its influence upon any type of systematic arrangement of plots. It must therefore be said that the present study confirms the views of Fisher, not only in the one special case, but in all other cases of systematic plot arrangements as well.

The practical field experimenter who desires the highest degree of scientific accuracy in his experiments, and especially in his estimate of the validity of eventual conclusions, should therefore be careful to avoid systematic plans and must choose his arrangements at random.

It is true that in one individual experiment the estimate of error, obtained in using a systematic arrangement, *may be* far better, *i.e.* more near to the true error, than the estimate obtained by random arrangement. Even then, however, the chance of obtaining a good estimate of

the error is diminished in using the systematic arrangement. And when, as is most often the case, a series of experiments relating to the same problem is carried out, there is no possibility of escaping the fact that systematic arrangements will give results deviating from the truth.

On the other hand, if practical considerations such as the increase of labour and of sources of mistakes, following the use of random arrangements, induce the experimenter to stick to one systematic plan, he should know that his results are not absolutely accurate.

If other sources of mistakes exist, which have not been or cannot be eliminated, the demands on full mathematical accuracy may perhaps be considered as exaggerated, like cutting wood with a razor. It is hoped that the present study contributes a means of measuring the importance of the errors introduced by using systematic plot arrangements. It must then be left to the individual worker to judge when the other conditions of his experiment have reached such a state of perfection that it is worth while to use the more elaborate methods of plot arrangement and of mathematical analysis.

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A STUDY ON THE EXTRACTION OF PAPAIN, THE ACTIVE DIGESTIVE PRINCIPLE FROM PAPAYA.

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THE Papaya tree is common in all parts of India and is grown also in the Philippine Islands, Federated Malay States, Java, Ceylon, and other tropical countries.

The present work was taken up at the Harcourt Butler Technological Institute, Cawnpore, U.P., India (1925–9), with a view to settle whether large-scale Papaya plantations could be profitably started in India for the supply of papain.

Ceylon has for several years been slowly and steadily developing an export trade in papain. The average price of the exports from Ceylon are:

1925	...	...	Rs. 7/1 per lb.
1926	...	...	Rs. 8/4 ,,
1927	...	...	Rs. 8/0 ,,

The above prices are low as compared with Merck's papain, which is quoted at Rs. 1/8 per ounce. This is a purified article with 80 per cent. proteolytic activity, while the article exported from Ceylon is only 40–50 per cent. active.

The wide variation in the price of papain renders it very essential for the successful running of a Papaya plantation to determine carefully the optimum conditions to obtain the maximum yield of juice and a product of a superior quality. To attain this object the effects of successive lancings, number of cuts, maturity of the fruit, different botanical varieties and varied manurial treatments were studied.

The practice generally followed to tap papain, the milky juice from the Papaya, is to incise the fruit longitudinally with a knife. The incisions should not be deep, but should be confined to the outer skin. The juice is collected in flat porcelain dishes and filtered through fine muslin, and then dried in the sun. The better way of drying the juice is to spread it in a thin layer in flat dishes and then place it in a hot-air chamber at a temperature not exceeding 35° C. The milky juice thus dried is then

ground and bottled. Sun drying is effective during summer, but in winter and the rainy season it takes too long to dry with the result that the juice begins to putrify; the utmost care must be taken to dry the juice as quickly as possible to keep its proteolytic activity intact.

The experiments were started in early April, a period when the fruits become mature, and were continued till about the same time next season. In the experiments the juice was collected in the morning, as is the usual practice, and continued for a week until the fruit ceased to give out any further juice. The juice was not filtered, but dried in a hot-air chamber and weighed.

I. Effect of successive lancings on the yield and proteolytic activity of different lancings.

It was found that the yield of papain goes on steadily decreasing in successive lancings until it dwindles almost to nothing. Each lancing includes the milky juice that exudes instantaneously on lancing the fruit, and also the dried juice that collects during the day and is scraped off next day before making the second incision. Table I contains a few examples:

Table I.

Lancings ...	1	2	3	4	5	6	7	Total
1. 1 fruit (gm.) (10 incisions at a time)	1·33	0·49	0·36	0·26	0·24	0·17	—	2·87
2. 6 fruits (gm.) (8 incisions at a time)	4·34	1·84	1·35	0·94	1·0	0·6	0·27	10·34
3. 44 fruits (gm.) (8 incisions at a time)	47·86	11·15	6·30	5·0	3·9	4·3	3·8	82·4
4. 9 fruits (gm.) (same plant as 3, 8 incisions at a time)	5·4	4·3	3·0	3·0	3·1	1·9	0·95	21·65

The proteolytic activity (*i.e.* albumin dissolving power) goes on decreasing in successive lancings, so that papain is most concentrated in the exudation that comes out in the first instance. The product obtained in Exp. 3 in Table I was analysed for proteolytic activity by the method described by Henry Caryl and Stanley[1].

[1] *Analyst* (1915), **40**, 57. Egg albumin solution is prepared by slightly beating the whites of six newly laid eggs, adding two volumes of 1 per cent. sodium chloride solution, filtering and diluting the filtrate so that 15 c.c. contain 0·4 gm. of coagulable protein. Of this standardised solution 15 c.c. are placed in an Erlenmeyer's flask and 1 c.c. papain solution added (prepared by dissolving 1 gm. papain powder in salt solution and diluting to 100 c.c. and leaving for ½ hour) and 9 c.c. of 1 per cent. sodium chloride solution added. The flask is transferred to a thermostat at 80° C. and, after 15 minutes' digestion, 1 c.c.

Table II.

Lancings ...	1	2	3	4		6	7
Yield (gm.)	47·9	11·5	6·3	5·0	3·9	4·3	3·8
Proteolytic activity (%)	63·6	62·05	58·82	49·56	36·21	—	—

Calculating the actual papain in the successive lancings from the yield and proteolytic activity, they are of the order:

Table III. *Actual papain in different lancings.*

Lancings ...	1	2	3	4	5	6	7	Total
44 fruits (gm.)	30·45	6·92	3·71	2·48	1·41	—	—	44·97

The yield of papain is thus about 1 gm. per fruit. Sometimes it so happens that after about 1 month the fruit, if not plucked from the tree, gives some residual juice, the yield of such juice diminishing in the successive lancings. The yield of juice tapped after 1 month from Exps. 3 and 4 in Table I is as follows:

Table IV.

Lancings ...	1	2	3	4	5	6	7	Total
53 fruits (gm.)	7·6	7·2	6·3	2·5	1·6	—	—	25·25

II. EFFECT OF THE NUMBER OF CUTS ON THE YIELD AND QUALITY OF THE JUICE.

The number of cuts given each time has a great influence on the yield and quality of the juice. Experimentally with four, eight and ten cuts, it was observed that the yield of juice obtained by using four cuts was the best, although there was no marked difference in the quality.

Table V.

Cuts ...	4	8	10
Yield per fruit (gm.)	2·8	1·1	1·33
Proteolytic activity (%)	69·9	63·6	65·3

III. EFFECT OF AGE ON THE YIELD AND QUALITY OF PAPAIN.

ᶜ Very small fruits yield a comparatively small amount of juice. The yield increases as the fruits become larger and the quality simultaneously goes on improving until it is full grown. Taking the smallest, medium

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$N/2$ acetic acid added and the flask transferred to a water bath at 100° C. where it is kept for 10 minutes. The undigested protein is collected on a tared filter paper, washed free from chlorides and then successively with 10 c.c. 95 per cent. alcohol and 10 c.c. ether and dried at 100° to 150° and weighed to constant weight. A control experiment shows how much protein is present in 15 c.c. of egg white solution.

and full grown fruit on the same plant, the yield in different lancings is as follows:

Table VI.

Lancings ...	1	2	3	4	5	6	7	Total
1. Smallest (gm.)	0·25	—	—	—	—	—	—	0·25
2. Medium (gm.)	0·75	1·8	0·5	0·45	0·3	—	—	3·6
3. Full grown (gm.)	1·8	1·7	0·7	0·35	0·35	—	—	4·9

A full grown fruit weighs about 500 gm. and takes about 3 months from formation to attain full growth. The proteolytic activity of the first lancings of the smallest, medium and full grown fruits was analysed, and it was found that the juice from the smallest was the least pure.

Table VII.

	Smallest	Medium	Full grown
Proteolytic activity (%)	44·5	49·6	50·5

Actual papain in the small, medium and full grown fruits is therefore in the following order:

Table VIII.

	Smallest	Medium	Full grown
First lancings only (gm.)	0·111	0·135	0·909

IV. Yield from Unripe Fruit.

One full grown unripe fruit, freshly plucked, was incised. It gave a very small yield of juice. Weight of the dry juice powder was 0·13 gm. against 1·33 gm., the normal quantity. It was lanced again but failed to exude further juice.

After lancing it was squashed and made into pulp. The juice was strained through cloth. On drying the yellowish liquid obtained in the sun a sticky viscous substance was obtained. On determining its proteolytic activity only 0·35 gm. of active substance was found to be present, while the normal weight of pure papain per fruit is about 1 gm.

On incision the fruit becomes stiff and fails to exude juice. It gets fully ripe after about 2 months.

The incisions, if not too deep, do not make any difference in the sweetness of the fruit. The latter may be sold in the market, although at a much reduced rate.

V. VARIATION IN THE YIELD OF PAPAIN FROM PAPAYA OF DIFFERENT BOTANICAL VARIETIES, AND GROWN UNDER DIFFERENT SOIL CONDITIONS.

Two plants were used to determine the total yield of papain throughout the year. The lancings were made in early April (1925), when the fruits, as a rule, become mature and ripe. One plant was situated in a shady ground and in fairly rich soil, the other was on a rather hard soil in a place exposed to sunshine. Both of these plants were of the Cawnpore variety.

Table IX. *April tappings. Tall Papaya in a shady and rich soil.*
(*Yield in gm.*)

	No. of fruits	Tappings							Total
		1	2	3	4	5	6	7	
Apr. 1926	44	47·9	11·2	6·3	5·0	3·9	4·3	3·8	82·4
	9	5·4	4·3	3·0	3·1	3·1	1·9	0·95	21·6
Residual from above:									
	53	7·6	7·2	6·3	2·5	1·6	—	—	25·2
Oct. 4, 1925	38	116·7	7·6	6·0	4·2	1·7	0·95	0·80	137·95
Oct. 11, 1925	21	13·5	6·4	4·2	4·0	3·5	2·5	3·9	38·1
Jan. 7, 1926	24	21·5	25·5	14·2	3·5	1·0	0·5	—	66·2
Mar. 23, 1926	24	19·2	8·3	6·3	6·1	3·1	2·0	—	45·0
May 7, 1926	12	5·0	3·0	1·8	0·7	0·6	0·5	—	12·6
Total	148								428·0

The total yield of papain during the whole season from the above plant was about 1 lb.

The other plant, which was situated in the Students' Garden, gave the following yields:

Table X. *Poor soil, exposed to sunshine.* (*Yield in gm.*)

	No. of fruits	Tappings							Total
		1	2	3	4	5	6	7	
Cawnpore 3									
Apr. 1924	1	1·33	0·49	0·36	0·26	0·24	0·17	—	2·85
Apr. 1924	6	4·34	1·84	1·35	0·94	1·0	0·6	0·27	10·34
Jan. 1926	31	7·2	4·1	4·5	8·2	5·5	1·7	2·1	33·3
Feb. 18, 1926	18	4·0	3·7	2·9	1·9	1·2	0·9	—	14·6
May 1926	30	—	—	—	—	—	—	—	30·0
Total	86								91·1

The yield varies according to the number of fruits borne by the plant. In the former case the number of fruits was 148, whilst in the latter case there were only 86 fruits. The vast difference in the yields of papain reduces the investigation in hand to a botanical problem depending mainly on the soil conditions. The first plant was particularly vigorous and was on a far better soil. Taking the experimental facts into consideration the yield of papain per tree varies from ¼ lb. to 1 lb. even

though the plants are of the same botanical variety. It depends entirely on the difference in soil conditions.

To settle the question whether one botanical variety would give a better yield than another, three known species, Cawnpore, Calcutta and Bombay, were taken and yearly record of the yield was made. The soil and other conditions were more or less similar as all the trees were situated in the same place, viz. Students' Botanical Gardens, Agricultural College, Cawnpore. The tappings were started in September 1925 and continued until the next September.

The record of the yield (in gm.) was as follows:

Table XI. *September tappings.*

| | No. of fruits | Tappings | | | | | | | Total |
		1	2	3	4	5	6	7	
Bombay 13									
Sept. 1925	83	54·0	10·0	8·0	4·5	3·7	5·8	3·7	89·6
Jan. 1926	28	11·7	9·0	7·7	6·7	6·4	6·6	5·2	53·2
Apr. 1926	28	—	—	—	—	—	—	—	53·2
Aug. 1926	75	21·5	10·5	10·0	7·1	5·8	2·7	—	52·6
Total	214								248·6
Bombay 6									
Sept. 1925	33	49·2	2·0	2·0	2·5	0·9	1·4	0·7	58·7
Jan. 1926	15	6·2	4·9	3·7	3·7	3·2	2·9	—	24·6
Apr. 1926	—	—	—	—	—	—	—	—	24·6
Aug. 1926	6	3·1	2·2	2·1	2·0	1·6	0·9	—	11·9
Total	79								119·8
Bombay 17									
Sept. 1925	7	2·7	0·5	0·8	0·3	0·2	0·1	0·1	4·8
Jan. 1926	4	1·3	0·7	0·6	0·4	0·2	—	—	3·8
				Tree fell in April.					
Calcutta 2									
Sept. 1925	20	21·0	0·8	0·1	0·05	0·17	—	—	22·0
Jan. 1926	8	0·9	1·1	0·6	0·6	0·2	—	—	3·4
Apr. 1926	9	—	—	—	—	—	—	—	3·4
Aug. 1926	45	6·0	2·5	2·2	1·3	—	—	—	12·0
Total	81								40·8
Cawnpore 17									
Sept. 1925	15	15·5	7·3	3·0	2·2	2·1	0·9	—	31·1
Jan. 1926	9	2·0	1·8	1·1	0·7	0·6	0·5	—	6·7
Apr. 1926	9	—	—	—	—	—	—	—	6·7
Aug. 1926	3	0·3	0·25	0·15	0·10	0·1	0·1	—	1·0
Total	36								45·5
Cawnpore 10									
Sept. 1925	50	44·7	10·5	7·2	1·7	0·7	0·4	—	106·2
Jan. 1926	15	5·7	4·4	3·3	3·0	2·9	3·0	—	22·3
Apr. 1926	15	—	—	—	—	—	—	—	22·3
Aug. 1926	16	2·3	1·5	1·0	0·9	0·5	—	—	6·1
Total	116								156·9
Cawnpore 7									
Sept. 1925	1	0·5	0·4	0·3	0·9	0·8	0·7	0·2	3·8
Jan. 1926	4	1·3	0·7	0·6	0·4	0·3	—	—	3·8
				Tree fell in April.					

The yield of papain varies with the number of fruits. The larger the number of fruits, the more is the yield.

Summarising the data obtained in Table IX to Table XI, we have:

Table XII.

No. of fruits	Total yield of papain (gm.)	Yield per 100 fruits (gm.)		No. of fruits	Total yield of papain (gm.)	Yield per 100 fruits (gm.)
(1) *Cawnpore variety—shade—rich soil.*				(2) *Cawnpore variety—sunshine—poor soil.*		
44	82·4	187·0		1	2·85	285·0
9	21·6	240·0		6	10·34	172·0
53	25·2	473·0		31	33·33	107·0
38	138·0	365·0		18	14·60	80·0
21	38·1	181·0		30	30·0	100·0
24	66·2	275·0		Total 86	141·14	Av. 148·8
24	45·0	204·0				
12	12·6	105·0				
Total 148	429·1	Av. 200·5				
(3) *Cawnpore variety—same soil as (2).*				(4) *Cawnpore variety—same soil as (2).*		
15	31·1	207·0		50	106·2	201·24
9	6·7	74·4		15	22·3	148·70
9	6·7	74·4		15	22·3	148·70
3	1·0	33·3		16	6·1	38·10
Total 36	44·9	Av. 97·02		Total 96	156·9	Av. 134·16
(5) *Bombay variety—same soil as (2).*				(6) *Bombay variety—same soil as (2).*		
83	89·6	108·0		33	58·7	180·0
28	53·2	190·0		15	24·6	164·0
28	53·2	190·0		15	24·6	164·0
75	52·6	70·0		6	11·9	198·3
Total 214	248·6	Av. 139·5		Total 69	119·8	Av. 176·6
(7) *Calcutta—same soil as (2).*						
20	22·0	110·0				
8	3·4	42·5				
9	3·4	42·5				
45	12·0	26·6				
Total 82	40·8	Av. 55·4				

Excluding (1), which is an unusually vigorous plant, the average yield for the Bombay variety per 100 fruits is 158·0 gm., while for the Cawnpore variety it is 126·6 and for the Calcutta variety 55·4.

The Bombay variety bears fruits which, when fully grown, are the largest in size, then comes the Cawnpore variety and last the Calcutta variety. The Bombay variety is long and oval, the Cawnpore yields roundish and medium fruits, and the Calcutta variety round and small fruits. The latter do not increase much in size, but ripen when still small as compared with the other two species.

In the experiments undertaken the Bombay variety gave the highest yield of papain per annum, viz. $\frac{1}{2}$ lb. For the Cawnpore variety the best yields were a little above $\frac{1}{4}$ lb. and for the Calcutta variety $\frac{1}{10}$ lb. The Cawnpore variety yielding 1 lb. in the April tappings was an unusually tall tree and must be an exceptional case. This, however, shows that

the growth of the Papaya plant depends much on the soil conditions, moisture, previous treatment with manures, exposure to sun, etc.

VI. Effect of manures on the yield of papain.

In Sanyal's[1] paper mention has been made of the following mixture of artificial manures which was found to give satisfactory results on Papaya trees.

Superphosphate	800 lb. per acre	
Sulphate of potash	315	,,
Nitrate of soda	250	,,
Sulphate of ammonia	190	,,
Black sand (volcanic ash)	445	,,

1 lb. of the above mixture was applied per tree at the planting time with success. The total nitrogen, potash and phosphorus were calculated from the above recipe. Organic nitrogenous manure in the shape of activated sludge (N 3·2 per cent.), cow-dung (N 2 per cent.), night soil (N 2·1 per cent.), castor cake (N 4·7 per cent.), neem (*Margosa*) cake (N 5·3 per cent.) and mahua (*Bassia latifolia*) cake containing a similar amount of nitrogen were also applied. The potash and phosphorus requirements were also added in the shape of potassium sulphate and superphosphate. Subsequently sand (good white Ganges sand) was also put in.

The effect of phosphorus and potash starvations was also studied.

Mahua cake containing the saponin mahurin and one from which the saponin had been removed were also experimented on.

In all the manurial experiments the same botanical variety, viz. the Cawnpore variety, was used. The following proportions in lb. of different manures were added per tree:

Table XIII.

Plot no.	Manurial treatment	Super-phosphate	Potas-sium sulphate	Sand	Acti-vated sludge	Cow-dung	Night soil	Neem cake	Castor cake	Mahua cake	Mahua cake (no mahurin)
1	Activated sludge	½	¼	¼	1½	—	—	—	—	—	—
2	Activated sludge (no potash)	½	—	¼	1½	—	—	—	—	—	—
3	Activated sludge (no superphosphate)	—	¼	¼	1½	—	—	—	—	—	—
4	Cow-dung	½	¼	¼	—	2	—	—	—	—	—
5	Night soil	½	¼	¼	—	—	2	—	—	—	—
6	Neem cake	½	¼	¼	—	—	—	1	—	—	—
7	Castor cake	½	¼	¼	—	—	—	—	1	—	—
8	Mahua cake	½	¼	¼	—	—	—	—	—	1	—
9	Mahua cake (no mahurin)	½	¼	¼	—	—	—	—	—	—	1

[1] *Agric. J. of India* (1921), **16**, 50.

VII. PREPARATION OF THE LAND.

The ground was ploughed 7 in. deep in August 1927, the big lumps were broken and stones hand-picked. Pits 2 ft. wide and 2 ft. deep were then dug out in lines at intervals of 7 ft. The mixture of manure, sand and earth was put in the top 6 in. of the pit, the rest being filled in with the finely ground earth.

Seeds were obtained from the Second Economic Botanist to the Government of the United Provinces. These were mixed with sand and earth and put in shallow trays containing broken brick at the bottom and leaf manure, sand and earth in the proportion of $1:\frac{1}{2}:1$. The sowings were made when rain set in on June 1, 1927. When the seedlings were 3–4 in. high they were transplanted to small pots (July 4, 1927). When the seedlings were 1 ft. high they were transplanted into the pits. The best time for transplanting is just after the rains, *i.e.* in the early part of September.

The plant starts bearing fruit about July just after the rains and goes on bearing fruit for 4 years continually, the yield in the first year being the highest. The tappings can be carried out every three months, *i.e.* four times in the year.

Too much water-logging round the roots of the newly planted seedlings in pits is harmful, leading to rotting.

The average height attained by the Papaya plants after 11 months from the date of sowing, treated with different manures, was recorded, which gave a rough measure of the effectiveness of a particular manure. The average height was obtained by measuring all the plants in the plots (about fifty in each sub-plot) and finding the mean.

Table XIV. *Average height of fifty Papaya plants after 11 months.*

1	2	3	4	5	6	7	8	9
Activated sludge	Cow-dung	Night soil	Neem cake	Castor cake	Activated sludge (no potash)	Activated sludge (no super-phosphate)	Mahua	Mahua (no mahurin)
3′ 11″	3′ 7″	3′ 11¼″	3′ 11″	4′	3′ 6″	3′ 8″	2′ 7″	3′ 11″

Table XV records the comparative yield of papain after the second lancing in the month of November 1928.

The total yield of papain would be approximately three times the yield at the second lancing.

It may be concluded from Tables XIV and XV that castor cake and night soil have the most favourable effect on the growth of the plant and papain-yielding capacity; neem cake and cow-dung come next in order.

Table XV. *Yield of papain (in gm.) from different manurial plots. Cawnpore variety—second lancing.*

Plot no.	Manurial treatment	No. of trees	Yield of papain	Yield of papain per 50 trees
1	Activated sludge	19	56	150
2	Activated sludge (no potash)	10	29	145
3	Activated sludge (no superphosphate)	10	75	375 (?)
4	Cow-dung	6	24	200
5	Night soil	12	68	283
6	Neem cake	18	82	228
7	Castor cake	14	60	214
8	Mahua	22	96	218
9	Mahua (no mahurin)	18	42	117

Although the rate of growth was not retarded by the activated sludge, the yield of papain was decidedly low as compared with the previously mentioned manures.

The effect of potash starvation was very marked, that of the superphosphate starvation being less so.

The deleterious effect of mahurin, the saponin in mahua, was only noticeable in the temporary retardation in the growth of the plant at the outset. This disappears after a time as can be seen from the yield of papain from the mahua-treated plot. As manure, mahua is as good as neem cake and cow-dung.

Changing of sex.

The male can be distinguished from the female plants about 10 months after planting, when flowers come out on the main stem of the female trees. With male plants, the flowers appear on the branches from the main stem. These latter do not bear fruit, and are cleared off. Some of the male plants change sex and bear fruit in the branches. Only three cases were found in the whole plantation covering 2¼ acres. On tapping these fruits, the yield of papain obtained was negligible. Hence from the point of view of papain-yielding capacity, the male plants which have changed to female are of very little use and should be cleared off along with other male plants.

VIII. CONCLUSIONS.

1. The first exudation of the milky juice from the Papaya gives the largest yield and the best quality of papain. The yield goes on diminishing in the successive lancings until the fruit fails to exude any further latex.

2. The method of four lancings at a time gives the highest yield of papain and does not exhaust the fruit quickly.

3. The yield of papain goes on increasing until it is at the maximum when the fruit is full grown but not ripe. This is the best time for tapping for papain.

4. A plucked fruit fails to exude any papain.

5. The Bombay variety of Papaya gives the best fruit and highest yield of papain. The Cawnpore variety is slightly inferior to the Bombay variety. The Calcutta variety is the worst as regards papain-yielding capacity.

6. Among the different organic fertilisers used in India, castor cake and night soil have the most favourable effect. Neem cake (*Margosa*) and cow-dung come next in order. Mahua (*Bassia Latifolia*) cake has a temporary deleterious effect, due to the presence of mahurin, which disappears after a time; otherwise it is as good as neem cake and cow-dung. Potash starvation has the most disastrous effect on Papaya, that of superphosphate starvation being not so marked.

INVESTIGATIONS INTO THE INTENSIVE SYSTEM OF GRASSLAND MANAGEMENT.

By THE AGRICULTURAL RESEARCH STAFF OF IMPERIAL CHEMICAL INDUSTRIES, LIMITED.

II. THE MINERAL CONTENT OF INTENSIVELY TREATED PASTURE AND A RELATIONSHIP BETWEEN THE NITROGEN AND PHOSPHORUS CONTENTS.

By A. W. GREENHILL AND H. J. PAGE.

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(With Five Text-figures.)

INTRODUCTION.

IN the first paper[1] of this series, data were presented concerning the chemical composition of pasture as grazed under the Intensive System of Grassland Management, in respect of the organic constituents of the herbage. The present paper deals with the mineral data obtained in the same series of investigations and with the relationship between the nitrogen and phosphorus contents of the herbage.

Recent work has emphasised the importance of an adequate supply and balance of minerals in animal nutrition. In investigations[2, 3, 4, 5, 6] at the Rowett Research Institute, Aberdeen, in 1924, Godden[4] showed that the herbage of poor grazing areas was markedly poorer in minerals, and to a less extent in nitrogen, than that of good grazing areas, and that, further[6], the mineral content may be modified appreciably by the application of mineral fertilisers. At the same time, Cruickshank[5] found the mineral content to show definite variations during the season, the lime, and to a less extent the phosphorus and nitrogen, increasing to a summer maximum and falling again later. The actual range of variation and the period of maximum value appeared to be influenced by the type of pasture and the nature of the grazing.

Woodman and his associates[7, 8, 9, 10] at the Cambridge University School of Agriculture, on untreated pastures cut at frequent intervals, have confirmed Cruickshank's finding[5] in respect of lime, whilst the

phosphorus and nitrogen constituents showed a generally opposite behaviour. Similar findings for intensive pastures are reported by Griffith and Phillips[11] at the University College of Wales, Aberystwyth, and by Archibald and Nelson[12] in America, at the Massachusetts Agricultural College; in the latter case, however, the lime content had not fallen again at the close of the investigation.

Important results have been obtained by Fagan and his associates [13, 14, 15, 16] at the Welsh Plant Breeding Station, Aberystwyth, on Italian rye grass mixtures and individual species of grasses and clovers under systems of frequent cutting. Considerable differences in mineral content were observed and it was shown that the mineral content may be modified by manuring. The grass leaf was found to be much richer in lime and nitrogen than the stem, while the phosphorus contents were generally similar. Both the leaf and stem showed marked seasonal variations in the mineral constituents and in nitrogen, and it was found that the variations in the leaf may be very different from those in the stem. The phosphorus and nitrogen contents of the leaf decreased with maturity while the lime content increased; in the stem all three decreased with maturity.

Outline of present investigations.

The investigations reported consisted of a study throughout the grazing season of the chemical composition of herbage as grazed under the Intensive System of Grassland Management, samples being examined from each paddock each time that the first-line stock were about to be turned on. Data were collected at three centres in two seasons, namely at Tollesby Farm, Marton, North Yorkshire, and at Melchet Court, Romsey, Hampshire, in 1927, and at the Imperial Chemical Industries, Limited, Agricultural Research Station, Jealott's Hill, Berkshire, in 1929.

The pastures at each centre received the normal Intensive System manuring with lime, phosphoric acid, potash and nitrogen, whilst at Tollesby Farm a non-intensive paddock, receiving only phosphoric acid, was included also and grazed rotationally with the intensive paddocks. A description of the centres and of the weather and management conditions, together with a note on the method of sampling, were given in the first paper[1] of this series. The weather conditions at the three centres differed considerably: in 1927 a normal spring was followed by an early drought and an exceptionally wet summer, the drought being more pronounced at Melchet Court than at Tollesby Farm, whilst 1929 provided one of the driest seasons on record.

MINERAL DATA.

The average mineral content of the herbage at the grazing stage, during successive periods of the grazing season, in respect of the constituents total ash, silica, lime and phosphoric acid, is shown graphically for the respective centres and seasons in Figs. 1 to 4, whilst the average seasonal values are given in Table I. The values for nitrogen are also included in Figs. 1 to 4; their relation to the phosphoric acid values is discussed later.

Table I. *Average seasonal mineral content of herbage at grazing stage.*
(On basis of dry matter.)

	Intensive paddocks			Non-intensive paddock 1927
Season	1927		1929	
Centre	Tollesby Farm (%)	Melchet Court (%)	Jealott's Hill (%)	Tollesby Farm (%)
Total ash	10·7	10·0	10·4	10·1
Silica (SiO₂)	2·57	1·64	3·86	2·48
Lime (CaO)	0·80	0·84	0·81	0·81
Phosphoric acid (P₂O₅)	0·83	0·80	0·56	0·77
Ratio lime : phosphoric acid ...	0·95	1·05	1·45	1·05

The main features of the results may be stated briefly as follows:

1. The lime content of the intensive pastures fluctuated appreciably during the season but the fluctuations exhibited generally no definite seasonal trend. The average seasonal content was practically identical in all cases.

2. The phosphoric acid content showed a definite variation during the season, falling during drought or the early summer flush period and recovering again after the drought or flush period respectively. The average phosphoric acid content was markedly lower in the dry season of 1929 than in the wet season of 1927.

3. The total ash and the silica contents showed fairly regular increases during the season in 1927, but somewhat irregular fluctuations in the dry season of 1929. The average seasonal ash contents were practically identical throughout; the average silica contents varied with centre and season.

4. Comparing the non-intensive paddock (a pasture of good quality receiving only phosphoric acid) with the intensive paddocks at Tollesby Farm in 1927, there was no appreciable difference in respect of the average silica and lime contents, but the intensive pastures contained a rather higher proportion of phosphoric acid and total ash than the pasture from the non-intensive paddock.

Discussion of mineral data.

The average value of about 0·8 per cent. for the lime content of the dry matter of herbage from intensive pastures, compares with average values of 1 per cent. and 1·1 per cent. given by Godden(4) and

FIG. 1.

GRAPHS SHOWING SEASONAL VARIATIONS IN
MINERAL AND NITROGEN CONTENTS OF HERBAGE
FROM INTENSIVE PADDOCKS AT GRAZING STAGE.
TOLLESBY FARM. 1927.

by Orr and Thomson(17) for good cultivated pasture in Great Britain, a difference which may possibly be explained by a slightly lower clover content of the intensive pastures. The high clover content of the herbage also probably accounts for the values of about 1·5 per cent. obtained by Woodman and his associates(7, 8, 9, 10) at Cambridge, on untreated

pastures under systems of frequent, close cutting. The lime content is also somewhat lower than that reported by Griffith and Phillips[11] for intensive pastures at Aberystwyth.

FIG. 2.

GRAPHS SHOWING SEASONAL VARIATIONS IN
MINERAL AND NITROGEN CONTENTS OF HERBAGE
FROM INTENSIVE PADDOCKS AT GRAZING STAGE.
MELCHET COURT - 1927.

The irregular fluctuations in the lime content of the herbage from intensive pastures, with a lack of any definite seasonal trend, together with the constancy of the average values irrespective of centre or season, are not readily explained, and are matters requiring further investigation. The results are at variance with the general finding of others, that the

lime content of pasture shows a regular seasonal variation, rising to a maximum summer value and then falling again.
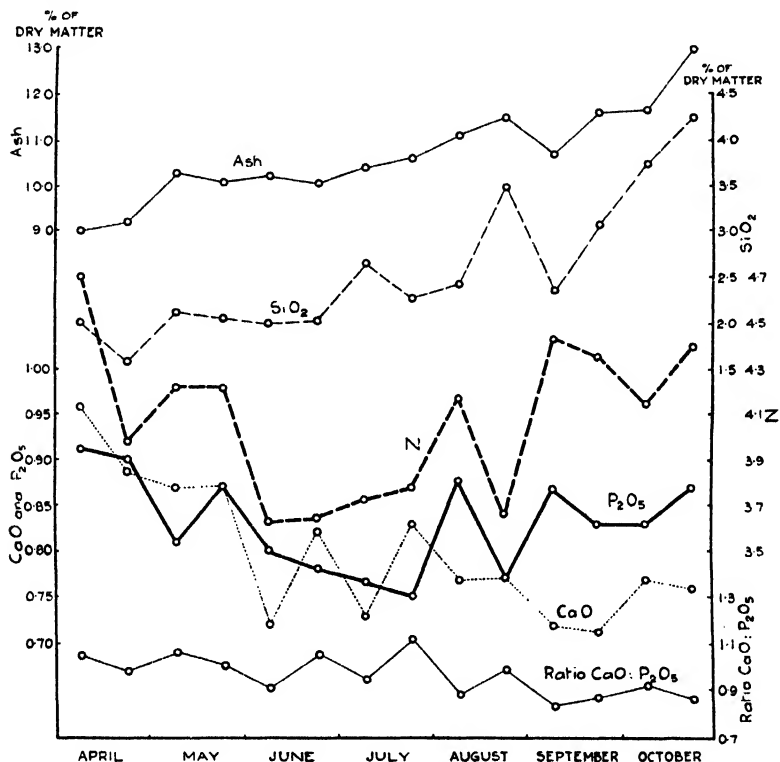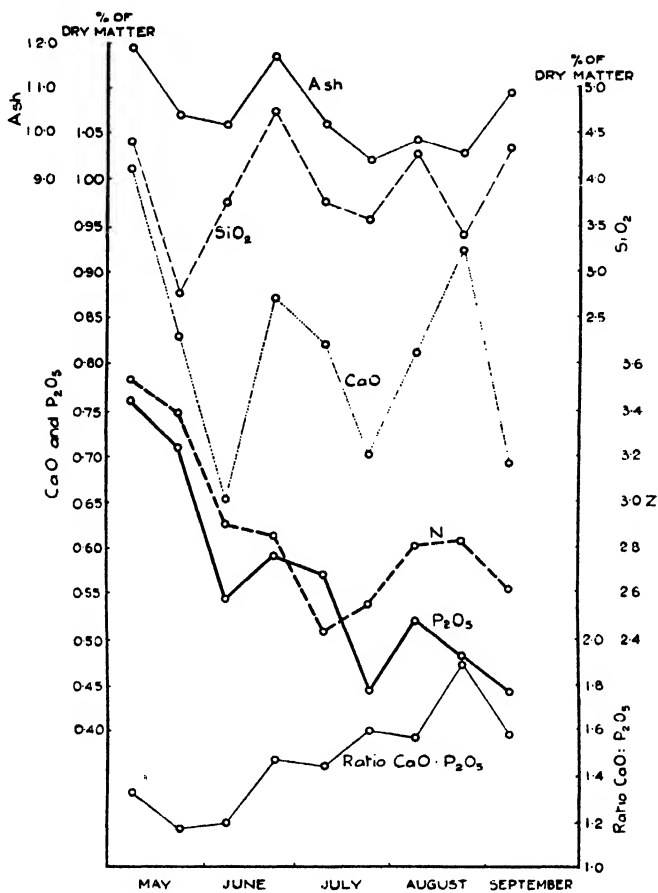

FIG. 3.

GRAPHS SHOWING SEASONAL VARIATIONS IN
MINERAL AND NITROGEN CONTENTS OF HERBAGE
FROM INTENSIVE PADDOCKS AT GRAZING STAGE.
JEALOTT'S HILL. 1929.

The phosphoric acid content of the intensive pastures is, except in the abnormally dry season of 1929, rather higher than the average values given by Godden(4) and by Orr and Thomson(17) for good cultivated

pasture, though a little lower than the values reported by Woodman and his associates(7, 8, 9, 10) and by Griffith and Phillips(11). The seasonal variations in the phosphoric acid content are discussed later.

The total ash and silica figures call for no particular comment.

The balance of lime and phosphoric acid in intensive pastures is of practical importance. In general, the ratio of lime to phosphoric acid varied according to the nutritive value of the herbage, the ratio rising
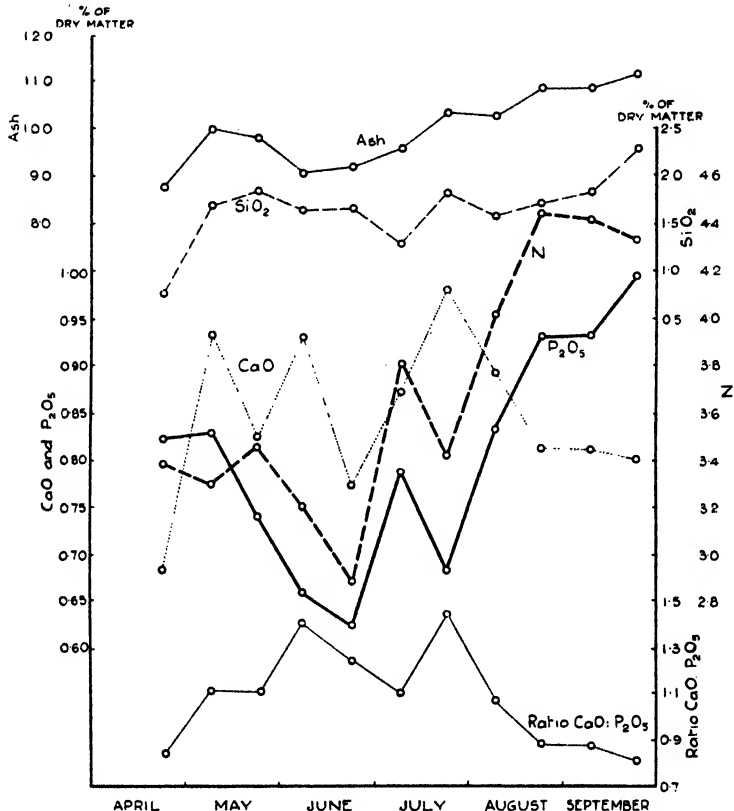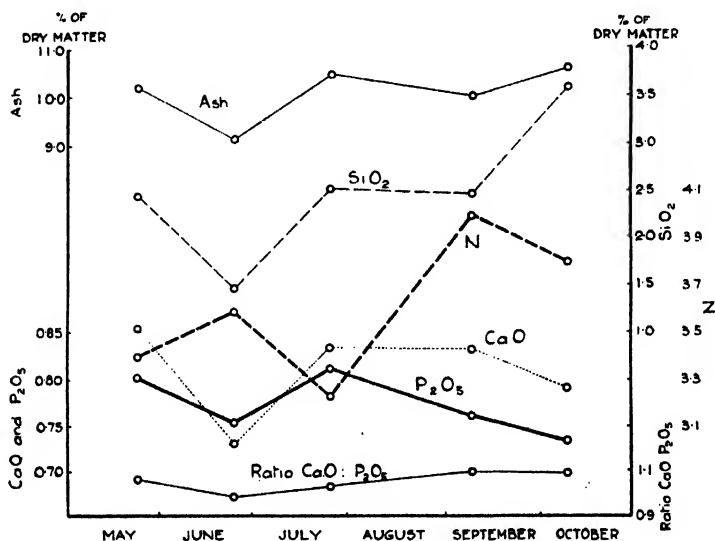

FIG 4.

GRAPHS SHOWING SEASONAL VARIATIONS IN
MINERAL AND NITROGEN CONTENTS OF HERBAGE
FROM NON-INTENSIVE PADDOCK AT GRAZING STAGE.
TOLLESBY FARM. 1927.

as the nutritive value decreased and vice versa. Under conditions of high nutritive value the ratio was about 1, but under conditions of lower nutritive value the ratio increased up to values approaching 2. Under dry conditions, therefore, intensive herbage becomes ill-balanced in respect of lime and phosphoric acid, being relatively low in the latter constituent, and it is recommended that at such times a supplementary ration should be fed which is relatively rich in phosphoric acid compared with lime. A similar finding and recommendation have been made by Woodman and his associates(7, 8, 9, 10).

RELATIONSHIP BETWEEN NITROGEN AND PHOSPHORUS CONTENTS OF
HERBAGE OF INTENSIVE PASTURE.

From Figs. 1, 2 and 3 it is evident that there is a marked association between the fluctuations in the content of nitrogen and of phosphoric acid of intensive pastures. The closeness of this association is shown by the correlation coefficients between the values for these two constituents at each centre, which are given in Table II, and by the correlation diagram for the results from all three centres (Fig. 5).

Table II. *Correlation coefficients*[1] *for phosphoric acid and nitrogen contents of herbage at grazing stage.*

	Intensive paddocks			Non-intensive paddock
Season	1927		1929	1927
Centre	Tollesby Farm	Melchet Court	Jealott's Hill	Tollesby Farm
Value of correlation coefficient, r...	0·786	0·883	0·851	0·787
Value of n	12	9	7	3
Value of r for $P=0·01$	0·661	0·735	0·798	0·959

The correlation coefficients are positive and highly significant for each intensive pasture, the values in all three cases being well above those required for a probability of 100 to 1 in favour of significance. On the non-intensive paddock at Tollesby Farm, however, a correlation is not evident.

A study of the pasture analysis data published by other investigators reveals evidence for the existence of a similar correlation in some cases, but not in all. Woodman and his associates[8] have called attention to a certain parallelism between the nitrogen and phosphoric acid contents of samples of pasture herbage obtained in cutting experiments at Cambridge. Similarly, Orr and Thomson[17] found high correlation coefficients between nitrogen and phosphoric acid in the analysis of miscellaneous samples of pasture herbage from various parts of Great Britain. The statistical significance of the correlations is, however, not stated. So far as the authors are aware, these are the only instances in the literature in which any reference is made to a relationship between the nitrogen and phosphorus contents of pasture herbage.

A study of the analytical data published by Griffith and Phillips[11] at Aberystwyth, by Archibald and Nelson[12] at Massachusetts and by

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[1] See *Statistical Methods for Research Workers,* by R. A. Fisher. Oliver and Boyd, London, 1928.

Cruickshank (5) at Aberdeen, however, affords further evidence. The Aberystwyth and Massachusetts data refer to intensive pastures, and in both cases there is a fairly close association between nitrogen and phosphorus. On a non-intensive pasture, however, as in the present
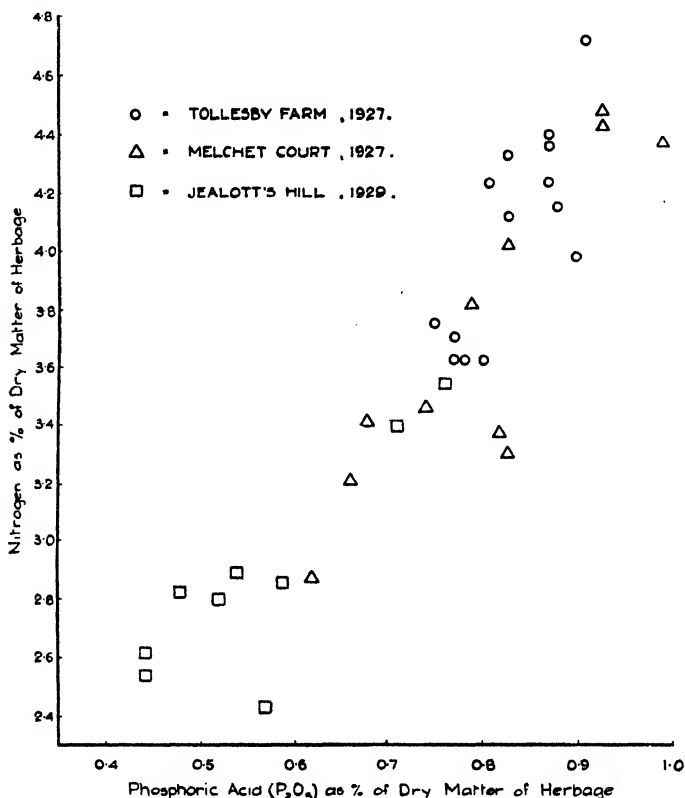
FIG. 5.

DIAGRAM SHOWING CORRELATION BETWEEN
NITROGEN AND PHOSPHORUS CONTENTS OF HERBAGE
FROM INTENSIVE PADDOCKS AT GRAZING STAGE.

investigations, Archibald and Nelson's data show no such association. Cruickshank's data from four untreated Scottish pastures show a positive correlation. Moreover, a further study of the data obtained by Woodman and his associates at Cambridge (7, 8, 9, 10) appears to show that, in pasture cuts at three-weekly intervals, there is a closer association between nitrogen and phosphorus than in cuts made at weekly or fortnightly

intervals. The data obtained by Fagan and his associates[16] at Aberystwyth are less easy to appraise in this connection, since they refer separately to leaf and stem from weekly and monthly cuts of Italian rye grass. There appears to be a closer relationship, however, between nitrogen and phosphorus in the stem than in the leaf.

The most definite feature emerging from this consideration is the fact that the positive N : P correlation exists in the herbage from intensive pasture, whilst on non-intensive pasture it is either less evident or absent. Until further work has been carried out on the subject, it is impossible to state what factors influence this correlation, favourably or adversely. There can, however, be no doubt about the closeness of the correlation in the authors' results, which is higher than that obtaining in any other published data that have been examined. The correlation is all the more striking in that it was obtained under three sets of pasture and climatic conditions of widely different character.

It is pertinent to enquire how far this correlation may be regarded as indicating a direct constitutional or functional relationship between nitrogen and phosphorus in the herbage, and, as a guide to further work, to consider the part played by various growth factors in their influence on the content of nitrogen and phosphorus respectively, in the herbage.

With regard to the first point, any change in the proportion of non-nitrogenous, non-phosphatic material in the herbage (such, for example, as the increase in the percentage of carbohydrates (fibre, etc.) which occurs during drought or the early summer flush period) would result in a simultaneous and opposite change in the proportions of both nitrogen and phosphorus, and, therefore, in a correlation between the percentages of these two elements which would not be indicative of any constitutional or functional relationship between them.

In this case, however, it would be expected that the percentage of other elements not present in the material in question would change in the same sense. The data for the calcium content of the herbage afford a means of testing this point. A glance at Figs. 1 to 3 shows that the changes in calcium content during the season are by no means positively associated with those of nitrogen and phosphorus. A detailed examination of the authors' analytical data and of those of the other investigators referred to above shows that for the intensive pastures there is a lack of positive correlation between nitrogen and calcium. Indeed, in some cases the positive N : P correlation appears to be accompanied by a negative N : Ca correlation. Positive N : P and N : Ca correlations are found together only in the results of Cruickshank[5] on unmanured

Scottish pastures. Thus it appears that the positive N : P correlation in intensive pasture herbage is not due simply to variations in the percentage of carbohydrates or other constituents devoid of both nitrogen and phosphorus.

The data under consideration refer essentially to young actively growing vegetative tissue, and any physiological significance that may ultimately be ascribed to the positive N : P correlation therein, will presumably apply specifically to the processes or products of the primary syntheses occurring in such tissue. Data regarding the variations in composition of reserve material of plants, such as those in seeds or tubers, which are the result of secondary processes involving the translocation of the primary products from the vegetative tissues, may have no decisive bearing on the significance of the relationships observed in the latter.

With regard to the possible effect of different growth factors in their bearing on the relative contents of nitrogen and phosphorus in the herbage, it is noteworthy that the contents of nitrogen and phosphates in the soil solution are affected very differently by variations in the moisture content of the soil. Since nitrates are wholly soluble, their concentration in the soil solution is inversely proportional to the moisture content. For a given soil, however, the phosphate concentration of the soil solution is approximately constant. Hence, in times of drought, the soil solution in contact with the plant roots contains relatively much more nitrate in relation to phosphate, as compared with times of ample moisture supply. Moreover, moderate drought conditions, and also the climatic conditions obtaining during the early summer flush period, are favourable to active nitrification, which will still further increase the disparity between the nitrate and phosphate contents of the soil solution at such times. The effect of such variations in moisture and climatic conditions on the calcium content of the soil solution is intermediate between that on nitrate and that on phosphate. In so far as the relative contents of nitrogen, phosphorus and calcium in the herbage is a reflection of the relative amounts of nitrate, phosphate and calcium in the soil solution, therefore, one would expect the variations in the nitrogen content to be more closely associated with those of calcium than with those of phosphate. That the reverse is the case in intensive pasture supports the view that the plant is not wholly dependent on the soil solution for its supply of phosphate. Another factor which may be of importance is the possibility that pasture plants may absorb their nitrogen, at least in part, in the form of ammonia.

The ratio of true protein (Stützer) to total (crude protein) nitrogen in the herbage was found to be practically constant throughout the season(1), so that the N : P correlation observed corresponds to a Protein : Phosphorus correlation. This suggests that phosphorus may play an essential part in the chain of processes culminating in protein synthesis in the green leaf. In view of the above considerations it is possible that phosphates are concerned either in the uptake of nitrogen by the plant root, and in the transport of the nitrogen from the root to the actual seat of protein synthesis, or in that synthesis itself. In either case it is further necessary to assume that the phosphate is immobilised, or at any rate unable to function for further transport or synthesis. If it were able to act over and over again, the correlation found would not exist. On the other hand, the correlation may indicate that phosphate uptake is in some way dependent on nitrogen absorption or translocation.

The further study of this problem thus involves an investigation of the relative effects of different levels of phosphate and nitrogen supply, in water and soil cultures, under varying growth conditions, on the composition of grasses at various stages of growth. Investigations on these lines are being continued in these laboratories. In regard to the practical bearings on nitrogenous and phosphatic manuring of pastures, a matter for further study is the relative effectiveness of applying phosphate along with the nitrogen in several dressings during the season, as compared with the more usual practice of applying all the phosphate early.

SUMMARY.

1. The mineral content of pastures farmed under the Intensive System of Grassland Management, and representing three to five weeks' growth, from three centres in two seasons, is reported.

2. The lime content fluctuated considerably during the season, but the fluctuations showed no definite seasonal trend. The average seasonal content was practically identical in all cases.

3. The phosphoric acid content showed a definite seasonal variation, falling during drought or the early summer flush period and recovering again after the drought or flush period respectively. The phosphoric acid showed consistently a very highly significant positive correlation with the nitrogen content of the herbage. The bearing of this correlation on the metabolism of the plant and on the phosphatic and nitrogenous manuring of pastures is discussed.

4. Variations during the season in the total ash and silica contents were less definite. The average seasonal ash contents were practically identical in all cases; the silica contents varied with centre and season.

5. A non-intensive pasture of good quality contained less total ash and phosphoric acid than the intensive pastures, though similar lime and silica contents.

6. The data are compared with other published pasture data.

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INVESTIGATIONS INTO THE INTENSIVE SYSTEM OF GRASSLAND MANAGEMENT.

BY THE AGRICULTURAL RESEARCH STAFF OF IMPERIAL CHEMICAL INDUSTRIES, LIMITED.

III. THE SEASONAL VARIATION IN THE MINERAL CONTENT OF PASTURE WITH PARTICULAR REFERENCE TO DROUGHT.

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(With One Text-figure.)

INTRODUCTION.

ORR[1] has pointed out that very little information is available on the mineral composition of pastures during drought. Klimmer and Schmidt[2] found a reduced intake of calcium and phosphorus by hay during severe drought, and Henry[3] states that periods of drought decrease the amount of mineral substances in the plant, particularly as regards calcium and phosphorus.

Woodman[4] examined the seasonal variation in the mineral content of pastures and found a sharp fall in the percentage of phosphorus during the month of June 1925, when practically no rain fell. The fall was arrested by rainfall and the phosphorus content again rose.

In the course of an experiment, samples of pasture were collected daily from early May to October and weekly from October to December. As the summer of 1929 was abnormally dry, the mineral analyses were carried out in order to determine the effect of drought on the composition of the pastures. It is thought that these figures will be of value, as there are extensive areas of low rainfall both in this country and abroad.

TREATMENT AND CONDITION OF PASTURE.

The flora of the experimental plots was rather poor, consisting of about 45 per cent. *Lolium perenne* (perennial rye grass), 25 per cent. *Agrostis* spp., 14 per cent. *Poa trivialis* (rough-stalked meadow grass),

with small percentages of *Holcus lanatus* (Yorkshire fog), *Festuca ovina*, *Alopecurus pratensis* (meadow foxtail) and *Trifolium repens* (wild white clover).

The area under experiment had been cut for hay in 1928 and received no artificial fertilisers or farmyard manure for that crop. On December 31 a dressing of 7 cwt. per acre of potassic basic slag, containing 14 per cent. P_2O_5 and 5 per cent. K_2O, was applied. This was followed on January 2, 1929, by a dressing of Buxton hydrated lime, 70 per cent. CaO. Two applications of sulphate of ammonia, at the rate of 1 cwt. per acre, on February 26 and at the beginning of June, and a dressing of nitro-chalk at the same level on November 27, completed the manurial scheme.

It was intended to sample the pasture at a stage when its nutritive value would be highest and of a length suitable for grazing. The aim was to allow a growth period of from three to four weeks between successive grazings. At no time throughout the year, however, was it possible to obtain pasture in this condition.

No growth occurred until early May, owing to the lack of rain and the very low ground temperatures experienced. The growth which then commenced was controlled by the rainfall which was very unevenly distributed during May, 1·57 in. falling in two hours on May 24. At the end of June there was insufficient grass for sheep grazing. In the early part of July the grass had "shot" and the flowering heads were removed by a mowing machine. There was then very little growth until the end of August, when there was sufficient grass for sheep to graze for a few days. The drought continued until the end of September and no further appreciable growth was obtained until mid-November, when a good sheep bite was available. The plots were grazed for five days from November 7. Very little further growth was evident for the remainder of the year.

The sampling of the grass commenced on May 11 and was continued daily until October 11. From that date until December 31 only one sample was taken weekly. The daily samples were examined for dry matter and nitrogen content in the course of another experiment, and weekly composite samples were formed from the dried material.

Mineral analyses were carried out on every second sample, so the data given represent the state of the pasture collected during every second week from May 11 until October 11. From that date the error of sampling is probably greater, as only one sample was taken for each fortnightly analysis.

METEOROLOGICAL DATA.

The abnormality of the year 1929 is shown in the summary of the meteorological records of the Station.

During January and February, frosts of an intensity unknown since 1895 were experienced. This was associated with a drought, only 1·675 in. of rain falling during the first three months of the year, instead of the average, about 6·3 in. This was followed by further droughts and periods of sunshine right up to October. During the last three months of the year the rainfall was extremely high, breaking records in all parts of England. Severe gales accompanied the rain.

Table I. *Meteorological conditions during* 1929.

Month ...	Jan.	Feb.	Mar.	Apr.	May	June
Rainfall in inches	1·06	0·59	0·025	1·07	3·15	0·745
Average rainfall per day	0·034	0·021	0·0008	0·036	0·102	0·027
No. of rainy days (0·01 in. or over)	10·0	7·0	2·0	11·0	12·0	10·0
Sunshine in hours	31·6	59·3	157·8	159·5	252·6	204·8
Average sunshine per day	1·0	2·1	5·1	5·3	8·1	6·82
No. of sunless days	19·0	11·0	4·0	2·0	2·0	2·0
Mean maximum daily temp. (° F.)	38·0	37·3	54·0	53·1	63·0	66·3
Mean minimum daily temp. (° F.)	29·0	25·5	32·0	32·6	43·0	46·4

Month ...	July	Aug.	Sept.	Oct.	Nov.	Dec.
Rainfall in inches	1·50	1·176	0·38	3·18	5·79	4·726
Average rainfall per day	0·048	0·038	0·013	0·103	0·193	0·152
No. of rainy days (0·01 in. or over)	8·0	10·0	2·0	13·0	20·0	25·0
Sunshine in hours	227·9	194·5	184·8	102·5	67·7	32·4
Average sunshine per day	7·3	6·27	6·2	3·3	2·26	1·04
No. of sunless days	1·0	0	1·0	4·0	8·0	15·0
Mean maximum daily temp. (° F.)	75·7	71·0	74·8	57·7	51·5	48·0
Mean minimum daily temp. (° F.)	50·0	51·0	50·7	42·2	36·9	37·4

The rainfall over the year, 23·39 in., however, is close to the average recorded for the Reading area from 1881 to 1915.

MINERAL ANALYSES.

Seventeen samples were collected in all, from May to December, and Table II gives the analytical data obtained. The seasonal variations are shown graphically in Fig. 1 in which the weekly rainfall is also plotted.

The soluble ash is defined as the ash soluble in boiling dilute hydrochloric acid. The phosphorus was determined directly on the dry material by the modified Pemberton-Neumann[5] method, the potassium as potassium perchlorate, the sodium by difference from the weight of mixed potassium and sodium chlorides and the calcium volumetrically by potassium permanganate.
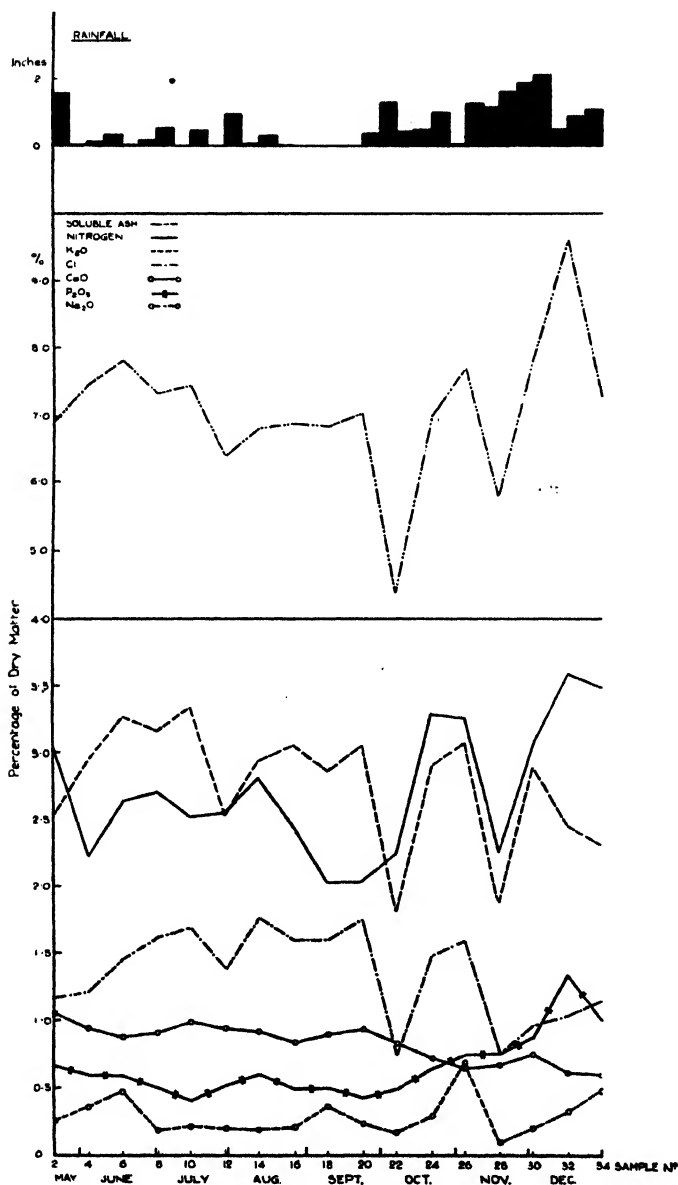
Fig. 1. Graph showing seasonal variation in mineral constituents.

Table II. *Mineral composition of pasture.*

Stated as percentages of dry material.

Date	Sample No.	Soluble ash	Calcium (CaO)	Phosphorus (P_2O_5)	Sodium (Na_2O)	Potassium (K_2O)	Chlorine (Cl)	Nitrogen (N)
21. v. 29	2	6·91	1·05	0·66	0·26	2·53	1·17	3·03
4. vi. 29	4	7·46	0·93	0·59	0·36	2·94	1·21	2·21
18. vi. 29	6	7·82	0·87	0·58	0·47	3·27	1·45	2·63
2. vii. 29	8	7·34	0·90	0·50	0·18	3·16	1·62	2·70
16. vii. 29	10	7·43	0·98	0·40	0·22	3·34	1·69	2·52
30. vii. 29	12	6·40	0·93	0·52	0·20	2·53	1·38	2·55
13. viii. 29	14	6·82	0·91	0·59	0·19	2·93	1·77	2·81
27. viii. 29	16	6·89	0·83	0·49	0·21	3·05	1·60	2·44
10. ix. 29	18	6·85	0·89	0·49	0·36	2·87	1·60	2·03
24. ix. 29	20	7·06	0·93	0·42	0·23	3·05	1·76	2·03
8. x. 29	22	4·37	0·82	0·48	0·17	1·81	0·73	2·23
22. x. 29	24	7·00	0·72	0·63	0·29	2·90	1·49	3·28
5. xi. 29	26	7·72	0·64	0·73	0·68	3·07	1·60	3·25
19. xi. 29	28	5·79	0·67	0·74	0·10	1·87	0·75	2·26
3. xii. 29	30	7·84	0·75	0·87	0·21	2·90	0·97	3·05
17. xii. 29	32	9·60	0·61	1·34	0·33	2·46	1·04	3·58
31. xii. 29	34	7·24	0·59	1·01	0·48	2·31	1·15	3·48

DISCUSSION OF RESULTS.

The individual constituents will be considered separately.

The *calcium* content during the drought period, *i.e.* up to the end of September, shows a slight decrease, the values varying from 1·05 to 0·83 per cent. CaO. From that date until the end of the year the fall is more marked, and the lowest value, 0·59 per cent. CaO, is recorded on December 31.

The *phosphorus* content also falls until the termination of the drought, the values ranging from 0·66 to 0·4 per cent. P_2O_5. With the onset of rain the percentage increases rapidly, the highest values being shown in December. It is noticed that during the drought period the highest and lowest points of the calcium curve coincide approximately with the lowest and highest points, respectively, of the phosphorus curve. Woodman[4] has noted this opposite variation in the lime and phosphorus contents during drought in the early part of the summer, but in his case this was associated with an increase in lime content.

The $CaO:P_2O_5$ ratio is very variable, very low values are apparent until October, when the ratio narrows and the phosphorus content finally exceeds that of the calcium.

The *potassium* content rises during June and July. The highest percentage of K_2O, 3·34 per cent., being recorded on July 16. A decrease occurs and the content remains about 3 per cent. in the droughty period in late August and September. Coincident with the rain which fell during

the first week in October the potash content showed a considerable drop in percentage. This decrease is also shown by the chlorine, and it is reflected in the soluble ash value. In order to see if this value was due to faulty sampling, the weekly samples collected immediately before and after No. 22 were examined for chlorine content. The results are No. 21 1·16 per cent. and No. 23 1·21 per cent. It will be seen that these values fall in line with the curve, showing that the drop is due to the conditions prevailing at that period. The cause of this depression is not apparent.

The growth increase, although no actual weights were taken, was not an inverse relation of a similar degree. Thus, the difference cannot be accounted for by the increased growth lowering the actual percentage of potash, presuming that no more potash is translocated by the roots or absorbed owing to the very dilute outside concentration. It would appear, therefore, that either the potash during this period was temporarily translocated to the roots, or that the heavy rain had leached out the potash from the surface of the leaves. Evidence of leaching from the leaves of plants is given by Mann and Wallace (7, 8), who show that considerable proportions of dry matter, ash and potash can be leached from many classes of fruit leaves. With certain varieties of leaves, over 90 per cent. of the potash is removed by leaching in two days. Whether this is also true of pasture leaves is a point which will need further investigation.

The potash content is quickly brought up to normal again when sufficient rain has brought into action the reserve of potash in the soil.

The recovery is apparent in the sample collected after a fortnight. The pasture was grazed from November 7–12 and difficulty was experienced in obtaining sample No. 28. The effect of the removal of the leafy portion of the pasture is shown in this sample, the percentage of all mineral constituents, except calcium and phosphorus, falling to a marked degree. The potash content rises again but decreases during December.

The *sodium* content rises at first and then falls in June and then remains fairly steady until the rain commences, when it rises. It falls after the grazing in November and then increases gradually until the end of the year.

The *chlorine* content of the pasture increases steadily until mid-July. It then fluctuates about 1·6 per cent. until the end of the drought. The highest values are recorded in August and September. The effect of the

rain and the grazing are felt in a manner similar to the potash. The values are lowest in late November and December.

The *soluble ash* varies throughout the year in a similar manner to the potassium, except for the last two samples collected in December, when the soluble ash rises.

The *nitrogen* content of the grass follows the rainfall to a marked degree—the lowest values being recorded during the very severe drought in September. A recovery is effected by the rain in October, and the grazing in November again decreases the percentage. After that date the values rise to the highest level of the season.

Recently Greenhill and Page (6) have shown that there is a marked positive correlation between the nitrogen and phosphorus contents of intensively treated pastures.

Their correlation coefficients, calculated from the analyses of pastures from three districts, give values lying between 0·786 and 0·981 which are well above the values required for a probability of 100 to 1 in favour of significance. These coefficients apply to the pasture collected during the grazing season.

From the data obtained in the present investigation, two series of correlation coefficients for various mineral constituents have been calculated. The first is from the pasture collected from May until the termination of the drought at the end of September, and the second is from that collected during October, November and December.

Table III. *Correlation coefficients for the mineral constituents of pasture collected during* 1929.

Period ...	21. v. 30–24. ix. 30	8. x. 30–31. xii. 30
Value for correlation coefficient, r		
P_2O_5 and N	+0·58	+0·68
P_2O_5 and Cl	−0·69	+0·01
K_2O and N	−0·21	+0·69
K_2O and P_2O_5	−0·52	+0·14
K_2O and Cl	+0·55	+0·83
CaO and K_2O	−0·41	−0·19
CaO and P_2O_5	+0·25	−0·73
CaO and Cl	−0·41	−0·37
CaO and N	+0·39	−0·32
Cl and N	−0·26	+0·68
Value of N	8·0	5·0
Value of r for $P=0·1$	0·549	0·669
$P=0·05$	0·632	0·754
$P=0·02$	0·715	0·833

It is evident that the relationship between nitrogen and phosphorus is not so marked during drought periods, the statistical significance only being of the order of 10 to 1 probability.

SUMMARY.

Seventeen samples of pasture collected from May to December, 1929, have been analysed for mineral constituents.

1. The results show that drought conditions seriously affect the mineral composition of pastures.

2. The calcium content showed a slight fall during the months of drought, and a more marked fall in the months of heavy rain from October to December.

3. The phosphorus content fell during the drought period and rose rapidly at the end of the year.

4. Potassium gave irregular results. It maintained a fairly high level during the drought and decreased during December.

5. The sodium content remained steady during the drought, and showed an increase in the rainy period at the end of the year.

6. Chlorine showed a definite increase during the drought and a decrease at the end of the year.

7. The nitrogen, throughout the year, was dependent on the rain to a very marked degree. Low values were obtained during the drought and high values at the end of the year.

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ON THE INFLUENCE OF SOIL TEMPERATURE ON THE GERMINATION INTERVAL OF CROPS.

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THE data collected under the Agricultural Meteorological Scheme of the Ministry of Agriculture have provided information for a number of years in a number of places on the dates of sowing and appearance above ground of wheat, winter oats, spring oats, spring barley, turnips and swedes.

The varieties reported on varied from place to place and year to year, but a study of the variation showed that in all except a few cases there was no varietal difference in "germination interval" as measured by the interval between sowing and appearance above ground.

The varieties which were anomalous in respect of germination interval have been omitted from this investigation[1].

[1] For each crop in each year, the variance in interval between sowing and appearance above ground was analysed into portions due to differences between varieties and differences within varieties. Of twenty-three cases so examined there were fifteen in which there was no significant difference between the variances within and between varieties, four cases in which the variance between varieties was significantly *lower* than that within, and four in which it was significantly higher. The four cases in which the variance between varieties was significantly higher were

(1) *Winter oats*, 1924–5. Here an examination showed that the higher variance between varieties was due to one variety, Potato—Victory grown at Craibstone only. This variety gave an interval of 15 days between sowing and appearance above ground, the mean value for all the other varieties being between 20 and 24 days.

(2) *Turnips*, 1924–5. Here two varieties, Aberdeen Green Top and Aberdeen Bullock Yellow, gave a mean interval of 6 or 7 days and the other two, Favourite Purple and Top Aberdeen, grown at Cockle Park only, gave an interval of 24 days, an undoubtedly significant difference.

(3) *Spring oats*, 1927. Here an examination showed that the higher variance between varieties was due to two varieties, Svälof Victory and Swedish King, grown at Rothamsted only. For these two the intervals were respectively 35 and 38 days, all the others being between 14 and 26 days.

(4) *Swedes*, 1928. Here out of ten varietal means all were between 10 and 14 days, except Lord Derby grown only at Aber, Model grown only at Wye and Caledonian grown only at Cockle Park. These gave intervals of 6, 7 and 17 days respectively.

Of these Model and Caledonian were grown in 1927 and 1926 when the interval was not outstandingly different from the average.

There was almost certainly a tendency for reporters to report different varieties sown

In some stations since 1925 and in all stations since 1926 4 in. and 8 in. soil temperatures have been observed.

Information was therefore available for a study of the relation between soil temperature and germination interval. Tables I–VI show the stations and varieties which have been used in this investigation.

Table I. *Winter wheat.*

	Year		
Station	1924–5	1926–7	1927–8
Craibstone	—	Standard Red (3 sowings)	Standard Red (3 sowings)
Sprowston	—	Cambridge Browick Yeoman II Squareheads Master Setter Fox Weibull's Standard Wilhelmina Iron III Little Joss	Yeoman II Wilhelmina Iron III Squareheads Master
Rothamsted ...	Standard Red	Million III Standard Red	Yeoman II Swedish Iron Million III Squareheads Master
Good Easter ...	—	Squarehead Master Yeoman II Little Joss Cambridge Browick Fox Wilhelmina Weibull's Standard Iron III	Yeoman II Wilhelmina Iron III Squareheads Master
Sutton Bonington ...	—	Yeoman I Little Joss Fox Crown	Yeoman I Fox Little Joss Crown
Wye	—	Yeoman II Yeoman I	Yeoman I Renown

in the same place on the same day as appearing above ground on the same day, whether this was strictly true or not, and this is very probably the explanation of the four cases in which the variance between varieties was significantly lower than that within; it is probable also that for this reason the variance between varieties is too low in other cases. But, although the variance is too low, there is no reason to suppose that the mean values are biased and this is the justification of the use of the data for the present purpose.

So as to maintain the apparent homogeneity of the material, the varieties which caused the varietal differences in cases (1), (2), (3) and (4) just mentioned have been omitted from the investigation.

This would in any case have been necessary in cases (1) and (2) because the corresponding soil temperature data were not available. For turnips there are in any case insufficient data for working out correlations.

Table I (*contd.*)

Station	Year		
	1924–5	1926–7	1927–8
Long Sutton ...	—	Squareheads Master Yeoman II Little Joss Cambridge Browick Fox Wilhelmina Weibull's Standard Iron III	Yeoman II Wilhelmina Iron III Squareheads Master
Aberystwyth ...	—	Standard Red (2 sowings)	—
Newton Abbot ...	—	Yeoman II Iron III Wilhelmina Cambridge Browick	—

Table II. *Winter oats.*

Station	Year			
	1924–5	1925–6	1926–7	1927–8
Craibstone	—	—	Grey Winter (3 sowings)	Grey Winter (3 sowings)
Sprowston	—	—	Grey Winter Marvellous Bountiful No. 914 Black Winter	Plentiful Grey Winter Marvellous Bountiful
Rothamsted ...	Grey Winter	—	Black Winter Grey Winter	—
Good Easter ...	—	Bountiful Black Winter Marvellous Grey Winter	Grey Winter Bountiful Marvellous No. 914 Black Winter Victory	Plentiful Grey Winter Marvellous Bountiful
Sutton Bonington ...	—	—	Bountiful Black Winter Marvellous White Winter Grey Winter	—
Wye	—	—	Bountiful	— .
Long Sutton ...	—	—	Bountiful Marvellous No. 914 Grey Winter Black Winter	—
Newton Abbot ...	—	Bountiful Black Winter Marvellous Grey Winter	—	—

Table III. *Spring oats.*

	Year			
Station	1925	1926	1927	1928
Craibstone... ...	—	Victory (2 sowings) Potato (2 sowings)	Potato (3 sowings) Victory (3 sowings)	Potato (2 sowings) Victory (2 sowings)
Sprowston ...	—	Golden Rain Victory Abundance Black Tartarian A. 88	Black Potato Thousand Dollar Abundance White Cross Victory Golden Rain A. 88	Victory Thousand Dollar Golden Rain A. 88
Rothamsted ...	Giant Eliza	—	—	—
Good Easter ...	—	Abundance Victory Golden Rain Black Tartarian	Golden Rain Thousand Dollar Abundance Black Potato White Cross Victory	Abundance Golden Rain Victory Thousand Dollar A. 88
Sutton Bonington	—	—	Abundance Cropwell Victory	Victory Abundance Cropwell Ascot
Sandford	—	—	King	—
Wye	—	Victory	Marvellous	Marvellous
Long Sutton ...	—	—	Abundance Victory Golden Rain White Cross Thousand Dollar Black Potato A. 88	Golden Rain Victory Thousand Dollar A. 88
Aber	—	—	—	Goldfinder Victory Record Black Tartarian
Aberystwyth	—	Record (2 sowings)	—	—
Newton Abbot ...	—	Abundance Thousand Dollar White Cross Black Potato Black Tartarian A. 88 Victory Golden Rain	Golden Rain Thousand Dollar Victory A. 88	—

Table IV. *Spring barley.*

Station	Year			
	1925	1926	1927	1928
Craibstone	—	Plumage Archer (2 sowings)	Plumage Archer (3 sowings)	Plumage Archer (4 sowings)
Sprowston	—	Plumage Archer Sunrise Spratt Archer Archer Goldthorpe Beaven's No. 25 No. 824 No. 825 New Cross Beaven's Archer	Spratt Archer New Cross Archer Goldthorpe No. 825 Beaven's No. 25 Sunrise Beaven's Archer No. 824	Spratt Archer No. 824 No. 825 Plumage Archer
Rothamsted	Plumage Archer	—	—	Standwell
Good Easter	—	Plumage Archer Beaven's Archer Sunrise Spratt Archer Archer Goldthorpe Beaven's No. 25 No. 833 No. 832	No. 824 No. 825 Archer Goldthorpe Beaven's No. 25 Spratt Archer Plumage Archer, 1924 Beaven's Archer Webb's Sunrise	Plumage Archer Spratt Archer No. 824 No. 825
Sutton Bonington ...	—	—	Spratt Archer Plumage Archer Beaven's 1924 Burton Malting Gold New Cross Triumphant	Spratt Archer New Cross Plumage Archer Sunrise
Wye 6	—	Plumage Archer	Plumage Archer Spratt Archer	Plumage Archer (2 sowings)
Long Sutton	—	—	—	Spratt Archer Plumage Archer No. 824 No. 825
Newton Abbot ...	4	—	Beaven's Archer Webb's Sunrise Spratt Archer Archer Goldthorpe	—

Table V. *Turnips.*

Station	Year		
	1926	1927	1928
Craibstone	Glenlogie .	—	Lettyton Green Top (2 sowings)
Wye	—	White	—

Table VI. *Swedes.*

Station	Year		
	1926	1927	1928
Craibstone ...	Inverquhomery	—	Aberdeenshire Prize
Sprowston	Best-of-all Smith's Green Top Purdy's Purple Top Gateacre Superlative Giant King Conqueror	Giant King Superlative Gateacre Conqueror Smith's Green Top Purdy's Purple Top Best-of-all	Best-of-all
Good Easter ...	Best-of-all Giant King Conqueror Smith's Green Top Superlative Gateacre	Gateacre Superlative Best-of-all Conqueror Giant King Smith's Green Top	—
Long Sutton ...	Best-of-all Conqueror Superlative Smith's Green Top Gateacre Giant King	Best-of-all Giant King Conqueror Smith's Green Top Superlative Gateacre	Best-of-all Giant King Superlative Gateacre Conqueror
Wye	Superlative	Model	—

Table VII. *Wheat.*

Correlation data between "germination interval" and mean daily soil temperature.

Station	Year	Interval (days)	Mean soil temperature (°F.)	
			4 in.	8 in.
Craibstone	1926–7	10·00	56·83	55·92
	„	26·00	41·96	43·41
	„	41·00	38·00	38·33
	1927–8	6·00	57·70	56·68
	„	6·00	45·96	46·27
	„	34·00	38·01	39·22
Sprowston	1926–7	29·33	42·10	42·82
	1927–8	30·00	42·82	43·42
Rothamsted	1924–5	25·00	42·72	—
	1926–7	26·50	45·30	46·14
	1927–8	16·00	51·38	50·99
Good Easter	1926–7	9·12	43·61	45·15
	1927–8	56·00	39·12	40·98
Sutton Bonington	1926–7	38·75	39·89	40·62
	1927–8	17·75	40·70	41·22
Wye	1926–7	18·50	46·29	46·39
	1927–8	11·00	51·38	51·02
	„	24·00	42·45	43·19
Long Sutton	1926–7	31·00	43·77	44·12
	1927–8	33·00	41·15	42·06
Aberystwyth	1926–7 ·	29·00	43·10	44·42
	„	27·00	41·79	42·65
Newton Abbot	1926–7	33·00	39·95	40·32

Table VIII. *Winter oats.*

Correlation data between "germination interval" and mean daily soil temperature.

Station	Year	Interval (days)	Mean soil temperature (° F.) 4 in.	Mean soil temperature (° F.) 8 in.
Craibstone	1926–7	11·00	56·56	55·74
	,,	26·00	41·96	43·41
	,,	44·00	37·82	38·19
	1927–8	8·00	57·13	56·21
	,,	13·00	47·67	47·65
	,,	40·00	38·25	39·38
Sprowston	1926–7	37·80	40·92	41·68
	1927–8	50·75	38·98	39·64
Rothamsted	1924–5	14·00	52·19	——
	1926–7	32·50	43·27	44·14
Good Easter	1925–6	62·00	37·97	39·15
	1926–7	28·50	44·26	45·51
	1927–8	55·00	39·08	40·90
Sutton Bonington	1926–7	33·20	41·29	42·04
Wye	1926–7	24·00	45·13	45·53
Long Sutton	1926–7	29·20	43·48	43·99
Newton Abbot	1925–6	40·00	37·91	38·53

Table IX. *Spring oats.*

Correlation data between "germination interval" and mean daily soil temperature.

Station	Year	Interval (days)	Mean soil temperature (° F.) 4 in.	Mean soil temperature (° F.) 8 in.
Craibstone	1926	26·00	40·78	40·34
	,,	17·00	45·90	44·89
	1927	21·00	41·85	41·05
	1928	25·00	40·98	39·82
	,,	26·00	40·72	39·62
Sprowston	1926	13·60	48·98	47·51
	1927	22·14	43·84	43·50
	1928	30·50	40·55	40·58
Rothamsted	1925	35·00	38·98	—
Good Easter	1926	23·00	44·14	43·58
	1927	19·67	45·95	45·23
	1928	28·00	43·33	42·78
Sutton Bonington	1927	24·00	44·05	43·47
	1928	27·00	42·76	42·11
Sandford	1927	14·00	53·49	51·34
Wye	1926	22·00	42·89	43·26
	1927	21·00	43·79	43·51
	1928	26·00	40·99	40·81
Long Sutton	1927	13·71	51·15	50·62
	1928	19·25	47·37	46·72
Aber	1928	10·00	55·68	53·29
Aberystwyth	1926	20·00	44·09	43·73
Newton Abbot	1926	17·62	51·48	50·35
	1927	20·50	47·37	46·61

Table X. *Spring barley.*

Correlation data between "germination interval" and mean daily soil temperature.

Station			Year	Interval (days)	Mean soil temperature (°F.)	
					4 in.	8 in.
Craibstone ...	...	...	1926	24·00	40·76	40·38
			,,	16·00	46·04	44·97
			1927	19·33	41·86	41·04
			1928	17·75	42·78	41·57
Sprowston ...	...	...	1926	11·78	49·11	47·61
			1927	14·00	48·64	47·37
			1928	27·00	42·19	41·82
Rothamsted	...	...	1925	15·00	39·28	—
			1928	12·00	46·11	44·53
Good Easter	...	...	1926	22·75	44·36	43·70
			1927	19·37	46·12	45·37
			1928	23·00	46·14	45·21
Sutton Bonington ...	...		1927	18·57	45·17	44·51
			1928	19·00	45·78	44·90
Wye ...	...	...	...	1926	18·00	43·84	43·60
			1927	17·00	45·80	44·57
			,,	10·00	52·60	49·57
			1928	22·50	41·46	41·12
Long Sutton	...	...	1928	18·00	47·35	46·59
Newton Abbot	...	...	1927	20·00	47·17	46·47

Table XI. *Swedes.*

Correlation data between "germination interval" and mean daily soil temperature.

Station			Year	Interval (days)	Mean soil temperature (°F.)	
					4 in.	8 in.
Craibstone ...	...	...	1926	15	46·19	45·10
			1928	10	46·82	45·93
Sprowston ...	...	...	1926	5	56·82	53·92
			1927	15	59·04	56·64
			1928	10	58·01	55·42
Good Easter	...	...	1926	9	58·75	55·27
			1927	15	58·71	56·19
Long Sutton	...	...	1926	6	62·53	60·21
			1927	26	60·68	59·46
			1928	13	59·68	58·83
Wye ...	...	...	...	1926	7	59·30	57·48
			1927	8	59·20	57·08

The convention was adopted that intervals which did not overlap were counted separately, but where they overlapped a mean interval was calculated. The mean soil temperature used was the mean of the 9 hr., 15 hr., 21 hr. readings. Where intervals overlapped an average of the daily mean temperature was calculated by totalling all the daily temperatures in the overlapping intervals and dividing by their number.

By this means enough pairs of observations to correlate were obtained. The data used are shown in Tables VII–XI. It will be seen that the number of pairs of observations varies from 12 to 24 for the different crops. (For turnips there were only four pairs of observations available and these were not sufficient in number to correlate.)

Both the regression of "germination interval" on 4 in. and 8 in. soil temperatures separately and the correlations were worked out. The former is naturally the more fundamental and useful for this purpose, as it gives the average decrease in "germination interval" for each degree increase in soil temperature.

On the basis of the data in Tables VII–XI we have the following results for mean interval, mean 4 in. and 8 in. soil temperatures and the variabilities of these quantities.

Table XII. *Means.*

Crop	Mean interval (days)	Standard error	Mean 4 in. soil temp. (° F.)	Standard error	Mean 8 in. soil temp. (° F.)	Standard error
Winter wheat ...	25·13	2·55	44·17	1·12	44·79	1·05
Winter oats ...	32·29	3·79	43·76	1·52	43·86	1·37
Spring oats ...	21·75	1·19	45·05	0·90	44·55	0·81
Spring barley ...	18·25	0·98	45·13	0·71	44·47	0·57
Swedes	11·58	1·66	57·14	1·49	55·13	1·40

Table XIII. *Variabilities.*

Crop	s.d.* of interval (days)	s.d. of 4 in. soil temp. (° F.)	s.d. of 8 in. soil temp. (° F.)
Winter wheat	12·23	5·39	4·93
Winter oats	15·63	6·26	5·49
Spring oats	5·81	4·41	3·89
Spring barley	4·36	3·18	2·48
Swedes	5·76	5·16	4·84

$$* \text{ Calculated from } \sqrt{\frac{S(x-x)^2}{n-1}}.$$

The standard errors do not seem unduly large when we realise that they include sources of variation due not only to varietal but also to soil and place differences.

The mean intervals for the two winter cereals do not differ significantly, while for the two spring cereals the difference is just significant. The mean interval for swedes is again significantly different from that of the other crops.

The mean soil temperatures for all the crops except swedes do not

differ significantly from one another. Nor are the mean 4 in. and 8 in. soil temperatures significantly different.

When we examine the variabilities by Fisher's "z" test we find that the variabilities of interval for the two winter-sown crops are not significantly different, nor are those for the spring cereals and swedes, but the last three differ significantly from the first two. The variability of 8 in. soil temperature for the spring barley is perhaps significantly smaller than for the other crops, apart from this soil temperatures exhibit no significant differences in variability.

The regressions and correlations are as follows:

Table XIV.

	Regression of "germination interval" on soil temperature (days per ° F.)				Correlation of "germination interval" and soil temperature (° F.)	
Crop	4 in.	Standard error	8 in.	Standard error	4 in.	8 in.
Winter wheat ...	− 1·69	0·33	− 1·89	0·36	− 0·75	− 0·74
Winter oats ...	− 2·18	0·31	− 2·40	0·39	− 0·87	− 0·86
Spring oats ...	− 1·17	0·13	− 1·17	0·14	− 0·89	− 0·88
Spring barley ...	− 0·81	0·26	− 1·21	0·31	− 0·59	− 0·68
Swedes... ...	− 0·02	0·35	+ 0·07	0·38	− 0·02	+ 0·06

Neither the regressions nor the correlations of any one crop with 4 in. and 8 in. soil temperatures differ significantly from one another.

For all the cereal crops the correlations are significant and, except for Spring Barley, high. The results for the winter-sown cereals are different from the spring-sown and we may summarise them by saying that the "germination interval" for winter wheat and oats is shortened by from 1·5 to 2 days for each increase of a degree F. in 4 in. or 8 in. soil temperature; for spring cereals the corresponding shortening is about a day.

Swedes clearly exhibit no correlation between "germination interval" and soil temperature.

This may well be because not soil temperature but soil moisture is the limiting factor.

EFFECT OF HYDROGEN PEROXIDE ON SOIL ORGANIC MATTER.

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TREATMENT of the soil with 6 per cent. hydrogen peroxide has been proposed by G. W. Robinson and J. O. Jones[1] as the basis of a method for estimating the approximate degree of humification of soil organic matter. The writer repeated this work using a different technique and found that whilst 6 per cent. peroxide did not attack cellulose—prepared from filter paper by successive treatment with 1·25 per cent. H_2SO_4 and 1·25 per cent. NaOH—in the absence of soil, there was a definite attack when soil was added under the conditions of the experiment—the attack varying with kind of soil. If cellulose added to a soil is attacked in this manner, it is reasonable to conclude that the so-called "structural organic matter" of the soil would also be attacked under the same conditions, and therefore the degree of the so-called "humification" thus determined would be invalid. This conclusion was drawn by W. O. Robinson[2] who, in fact, used 15 per cent. peroxide for the determination of the total organic matter in soils. Furthermore, it has been found that with the same quantity of soil and varying quantities of the same concentration of peroxide, a different "degree of humification" is obtained in each case.

EXPERIMENTAL.

Before presenting the results of the present investigations, it is necessary to give the technique followed.

When a soil is treated with peroxide, oxidation of the organic matter takes place and is usually accompanied by frothing of the liquid. The resulting products are carbon dioxide and various organic acids which are soluble and can be removed by filtration. The residue contains organic matter which has not been acted on by the peroxide. Its content of carbon and nitrogen can be determined after drying.

The method employed for the determination of "residual carbon" and "residual nitrogen" is as follows: 1 to 6 gm. of soil—100 mesh sample—are weighed into a 400 c.c. beaker and boiled for about 20 min. with 60 c.c. H_2O_2 and then with about 40 c.c. of water to small bulk.

The product is filtered through hardened filter paper and washed several times with boiling water. The residue is transferred to a 150 c.c. beaker, dried, ground in a mortar, transferred to a Kjeldahl flask, and the carbon and nitrogen estimated by the method of Robinson, McLean, and Williams[3].

Two Welsh carbonate-free soils were treated with 60 c.c. of 6 per cent. peroxide and repeated applications of 40 c.c. of 6 per cent. peroxide as above. The weight of soil used in each case was 4 gm., as it was found that the content of carbon and nitrogen in the residue was a convenient quantity to handle by the above method. The Madryn soil contains 4·31 per cent. carbon and 0·39 per cent. nitrogen; the Bodrwyn soil, 5·07 per cent. carbon, and 0·505 per cent. nitrogen. The results are given in Table I.

Table I. *Percentages of total carbon and nitrogen oxidised by varying amounts of 6 per cent. peroxide.*

Volume of 6% peroxide in c.c.	Residual				Percentages of total C and N oxidised				C/N ratio of residual organic matter	
	Percentage C in soil		Percentage N in soil		C		N			
	Madryn	Bodrwyn	Madryn	Bodrwyn	Madryn	Bodrwyn	Madryn	Bodrwyn	Madryn	Bodrwyn
60	0·605	0·640	0·044	0·058	86·0	87·4	88·7	88·5	13·8	11·0
100	0·468	0·480	0·042	0·057	89·1	90·5	89·2	88·7	11·1	8·4
140	0·404	0·360	0·043	0·054	90·6	92·9	89·0	89·3	9·4	6·7
180	0·343	0·265	0·042	0·056	92·0	94·8	89·2	88·9	8·2	4·7
220	0·287	0·235	0·044	0·054	93·3	95·4	88·7	89·3	6·5	4·4
260	0·269	0·210	0·042	0·054	93·8	95·9	89·2	89·3	6·4	3·9
400	0·242	0·213	0·043	0·056	94·4	95·8	89·0	88·9	5·6	3·8
500	0·226	0·212	0·042	0·056	94·8	95·8	89·2	88·9	5·4	3·8

It will be seen from Table I that oxidation of organic carbon proceeds with repeated additions of peroxide until a point is reached at a volume of about 260 c.c. when 94 to 96 per cent. of the total carbon has been removed, after which practically no further oxidation takes place with the particular concentration of peroxide used. In the case of compounds containing nitrogen, however, *there is no further oxidation by repeated additions of peroxide beyond the amount that has occurred with the initial volume of 60 c.c.* This, in effect, means that repeated additions of peroxide oxidise non-nitrogenous organic compounds only. Now it is well known that humified organic matter contains nitrogen; therefore repeated applications of 6 per cent. peroxide do not oxidise any humified organic matter beyond what has taken place with the initial volume of 60 c.c. This volume, therefore, seems to be sufficient to decompose the organic compounds containing both carbon and nitrogen. On the above showing, one might, therefore, differentiate the organic matter of soils into two

classes, viz. "oxidisable organic matter"—consisting of nitrogenous and non-nitrogenous material oxidisable simultaneously by peroxide—and "residual organic matter." The latter group contains nitrogenous organic matter which is not oxidised by repeated applications of 6 per cent. peroxide together with nitrogen-free organic matter which is oxidisable under these conditions.

It was also found that cellulose added to a soil was attacked by 6 per cent. peroxide to an appreciable extent, viz. 12·1 and 10·4 per cent. in the case of Madryn and Bodrwyn soils respectively. It is evident, therefore, that this concentration of peroxide is too high if an attack on the so-called "structural organic matter" is to be avoided.

The following questions now arise: Would any concentration of peroxide lower than 6 per cent. decompose completely the oxidisable associated nitrogenous and non-nitrogenous compounds, and if so, would the concentration found to be sufficient for this purpose have any effect on added cellulose?

To ascertain the lowest strength of peroxide necessary to decompose that portion of the organic matter here designated "oxidisable organic matter," the Madryn and Bodrwyn soils referred to in Table I were used and the results are presented in Table II.

Table II. *Percentages of total carbon and nitrogen oxidised by varying concentrations of peroxide.*

| Concentration of peroxide (%) | Residual | | | | Percentages of total C and N oxidised | | | | C/N ratio of residual organic matter | |
| | Percentage C in soil | | Percentage N in soil | | C | | N | | | |
	Madryn	Bodrwyn	Madryn	Bodrwyn	Madryn	Bodrwyn	Madryn	Bodrwyn	Madryn	Bodrwyn
6	0·605	0·640	0·044	0·058	86·0	87·4	88·7	88·5	13·8	11·0
5	0·627	—	0·042	—	85·5	—	89·2	—	14·9	—
4	0·675	0·823	0·044	0·058	84·3	83·8	88·7	88·5	15·3	14·2
3	0·792	0·910	0·042	0·060	81·6	82·1	89·2	88·1	18·9	15·2
2	0·982	1·170	0·053	0·068	77·2	76·9	86·4	86·5	18·5	17·2
1	1·540	—	0·111	—	64·3	—	71·5	—	13·9	—

It will be noted from Table II that with decreasing concentrations of peroxide the "residual carbon" increases regularly but *the "residual nitrogen" remains constant with concentrations of 6 to 3 per cent. peroxide and thereafter increases*, which shows that with the two soils examined, 3 per cent. is as effective in decomposing the organic compounds containing carbon and nitrogen as 6 per cent. peroxide and repeated applications of 6 per cent. peroxide. Further, it was found by a separate experiment that there was practically no attack on cellulose added to the soil by 3 per cent. peroxide or lower concentrations. The figures obtained for the attack of cellulose carbon for the Madryn and Bodrwyn

soils with 3 per cent. peroxide were 3·5 and 3 per cent. respectively, which are near the limits of experimental error. The attack of cellulose carbon added to a soil was determined by treating the soil plus 0·15 gm. cellulose with the peroxide in the ordinary manner and estimating the residual carbon. The total carbon found minus the residual carbon of the soil by itself gives the carbon recovered from the cellulose.

On the strength of the results obtained with the Madryn and Bodrwyn soils and those for several other soils for which exhaustive figures were determined, it was thought that 3 per cent. would be a suitable concentration of peroxide to differentiate the organic matter of soils into the two categories above mentioned. This view was upheld by a consideration of the C/N ratio of the "residual organic matter" obtained with varying concentrations of peroxide. These ratios are given in Tables I and II and it is seen that this ratio increases gradually with decreasing concentrations of peroxide. When more and more of the carbon alone has been removed, as in the case of higher concentrations, low ratios are, of course, obtained which clearly shows that the "residual carbon" has been attacked. One would naturally expect that the C/N ratio of the "residual organic matter" would be at least about 10, this figure being the mean for a large number of British soils for the total organic matter[4], whereas ratios as low as 3·8 were obtained for the "residual organic matter," with repeated attacks of 6 per cent. peroxide.

Since the content of organic matter in soils varies considerably and since the ultimate object of this investigation is to determine the percentage of "oxidisable organic matter" whose carbon and nitrogen are oxidised simultaneously and, if possible, correlate this with fertility and other factors, it is obviously necessary to consider whether variable amounts of organic matter would affect this percentage if the volume of peroxide is kept constant. Results are given in Table III.

The results in Table III demonstrate that the percentage of the total carbon oxidised by 3 per cent. peroxide decreases with increasing amounts of organic carbon used in the determination. It was, therefore, decided in future experiments to ensure that approximately equal amounts of carbon were used, in order to secure comparability among the results obtained. Approximately 0·1 gm. of carbon was fixed upon as the desirable amount under the conditions of experiment. It is possible that the use of smaller amounts of organic matter might have resulted in greater oxidation, but this would have entailed serious experimental errors.

Having decided on the weight of soil for a determination, it is now

necessary to consider if a volume of 60 c.c. of 3 per cent. peroxide (a convenient quantity to handle in a 400 c.c. beaker) is sufficient to decompose completely the oxidisable organic compounds containing carbon and nitrogen and so ascertain if an end-point is reached by this treatment. The results for a number of soils indicated that there was no significant increase in the percentage of the total carbon oxidised, and at the same time the percentage of the total nitrogen oxidised remained constant as was anticipated. It should, however, be mentioned at this stage that only non-carbonate soils behaved in this manner. Results for carbonate soils are given in a subsequent table.

Referring back to Tables I and II it will be noted that 4 gm. of soil equivalent to 0·172 gm. of carbon in the Madryn soil and 0·203 gm. of carbon in the Bodrwyn soil were used in all the determinations. In view of the results presented in Table III these quantities of carbon are considerably in excess of the amounts indicated to be requisite, and would presumably affect the percentages of the total carbon and total nitrogen oxidised. It was accordingly decided to repeat the investigations on the Madryn and Bodrwyn soils as well as on a number of other soils. These results are presented in Table IV but, for economy of space, the percentages of residual carbon and nitrogen in the soil are omitted. Only the percentages of the total carbon and nitrogen oxidised by peroxide are given, which, of course, can be calculated from the residual carbon and residual nitrogen figures. The attack on cellulose was determined in a parallel series of experiments.

A comparison of the figures in Table IV for the Bodrwyn and Madryn soils with the figures in Table II shows that there is a slightly higher percentage of the total carbon oxidised by the peroxide than when a smaller quantity of soil had been used, but the nitrogen figures are remarkably constant. In all the soils examined, the nitrogen figures are nearly constant for all the concentrations of peroxide down to 3 per cent., and in some cases for lower concentrations, but the carbon figures go down fairly regularly with decreasing concentrations.

With regard to the attack on cellulose carbon added to the soil, it will be seen that this varies considerably from soil to soil and for the different concentrations of peroxide in each soil. In the case of the Llysfasi, Herefordshire and Broadbalk soils there is no appreciable attack with 5 per cent. peroxide, whereas in the other three cases the attack does not become negligible until a concentration of 2 per cent. is reached. The former were found to contain carbonate, whilst the latter do not. On a critical examination of all the factors concerned,

Table III. *Effect of varying the quantity of soil, using a constant volume of 3 per cent. peroxide.*

| Weight of soil (gm.) | Weight of C (gm.) | | | Residual | | | | | | Percentage of total C and N oxidised | | | | | |
| | | | | Percentage C in soil | | | Percentage N in soil | | | C | | | N | | |
	Madryn	Bangor	Aber 6	Madryn	Bangor	Aber 6	Madryn	Bangor	Aber 6	Madryn	Bangor	Aber 6	Madryn	Bangor	Aber
1	0·043	0·068	0·028	0·502	1·221	0·433	0·046	0·068	0·048	88·4	82·0	84·4	88·2	88·5	83·4
2	0·086	0·136	0·056	0·563	1·482	0·465	0·044	0·087	0·053	86·9	78·2	83·3	88·7	85·3	81·7
3	0·129	0·204	0·083	0·668	1·954	0·483	0·044	0·107	0·059	84·5	71·3	82·6	88·7	81·9	81·7
4	0·172	0·272	0·111	0·802	2·476	0·570	0·063	0·150	0·068	81·4	63·6	79·5	86·4	74·6	79·7
5	—	0·340	0·139	—	2·880	0·616	—	0·185	0·071		57·6	77·8	—	68·6	76·6
															75·5

Madryn soil contains 4·31 % total C and 0·39 % total N.
Bangor " " 6·80 % " 0·59 % " "
Aber 6 " " 2·78 % " 0·29 % " "

Table IV. *Effect of varying concentrations of peroxide on soil organic matter and on cellulose added to the soil.*

| Concentration of peroxide (%) | Bodrwyn | | | Llysfasi | | | Herefordshire 92 | | | Aber 2 | | | Madryn | | |
| | Percentage of total C and N oxidised | | Attack on added cellulose | Percentage of total C and N oxidised | | Attack on added cellulose | Percentage of total C and N oxidised | | Attack on added cellulose | Percentage of total C and N oxidised | | Attack on added cellulose | Percentage of total C and N oxidised | | Attack on added cellulose |
	C (%)	N (%)	C (%)	C (%)	N (%)	C (%)	C (%)	N (%)	C (%)	C (%)	N (%)	C (%)	C (%)	N (%)	C (%)
6	92·4	88·1	22·7	80·7	80·5	1·6	89·3	78·7	6·7	91·0	83·8	26·1	90·0	88·3	26·6
5	92·4	87·7	21·0	78·6	80·5	Nil	88·9	78·3	3·5	90·5	84·5	21·6	90·0	88·7	19·7
4	91·6	88·1	13·8	78·6	80·5	1·6	87·2	79·2	Nil	90·0	86·5	16·7	89·8	89·0	12·6
3	90·6	88·1	6·0	75·2	80·5	3·0	84·7	79·2	Nil	89·2	86·0	9·5	88·6	88·6	7·5
2	88·6	88·3	3·1	67·0	71·3	1·2	81·4	79·2	Nil	87·0	85·3	2·8	86·3	89·0	5·0
1	81·1	87·7	0·8	—	—	—	70·3	72·2	Nil	81·4	84·3	Nil	79·6	87·5	2·0

| | Broadbalk, F.Y.M. plo[t] | | |
| | Percentage of total C and N oxidised | | Attack on added cellulose |
	C (%)	N (%)	C (%)
6	84·9	87·6	3·9
5	84·0	87·6	1·5
4	81·9	85·8	Nil
3	77·8	84·3	Nil
2	68·2	74·6	Nil
1	56·7	39·6	Nil

Soil	Total C (%)	Total N (%)	Weight of soil (gm.)	Weight of carbon (gm.)
Bodrwyn	5·07	0·51	2	0·102
Llysfasi	2·33	0·24	4	0·093
Herefordshire 92	2·42	0·23	4	0·097
Aber 2	3·97	0·40	2	0·079
Madryn	4·31	0·39	2	0·086
Broadbalk F.Y.M.	2·70	0·26	3	0·081

the writer considers, as a first approximation, that treatment of the soil with 3 per cent. peroxide differentiates soil organic matter into the categories aforementioned.

COMPARISON OF FERTILE AND INFERTILE SOILS.

It was next decided to investigate the behaviour under 3 per cent. peroxide treatment of soils of varying fertility. Results are given in Table V.

Table V. *Percentages of total carbon and nitrogen oxidised by 3 per cent. peroxide in soils of varying fertility.*

| | | Total | | Oxidised by 3 % peroxide | | | | | |
| | | | | C (%) | N (%) | Percentage of total | | | |
Soil	Fertility	C (%)	N (%)			C	N	CaCO$_3$ (%)	pH
Broadbalk, F.Y.M. plot, Rothamsted	High	2·70	0·26	2·091	0·219	77·8	84·3	3·6	Alkaline
Broadbalk, no manure plot, Rothamsted	Low	1·06	0·12	0·585	0·076	55·2	63·4	4·1	Alkaline
Stackyard, F.Y.M. plot, Woburn	High	1·51	0·15	1·290	0·128	85·4	85·3	—	6·2
Stackyard, no manure plot, Woburn	Low	0·90	0·10	0·780	0·075	86·7	75·0	—	5·9
Barnfield, F.Y.M. plot, Rothamsted	High	2·85	0·28	2·237	0·227	78·5	81·2	2·2	Alkaline
Barnfield, no manure plot, Rothamsted	Low	0·71	0·11	0·270	0·046	38·0	41·9	3·0	Alkaline
Herefordshire, 139	High	1·62	0·19	1·301	0·144	80·3	75·8	0·35	Alkaline
Herefordshire, 331	Low	1·32	0·15	0·870	0·099	65·9	66·0	0·8	Alkaline
Padi soil, Siam, B.A.S. 9	High	6·28	0·54	5·817	0·434	92·6	80·4	—	4·0
Padi soil, Siam, B.A.S. 3	Low	1·63	0·18	1·441	0·113	88·4	62·8	—	4·5
Aber, 2	High	3·97	0·40	3·540	0·344	89·2	86·0	—	6·1
Aber, T 12	High	3·26	0·39	2·683	0·328	82·3	84·1	—	5·7
Aber, 6	Moderate	2·78	0·29	2·338	0·228	84·1	78·7	—	6·2
Aber, B 12	Low	2·71	0·31	2·257	0·255	83·3	82·3	—	6·0
Herefordshire, 92	High	2·42	0·23	2·050	0·182	84·7	79·2	0·1	7·2
Herefordshire, 26	Low	1·71	0·19	1·373	0·146	80·3	77·0	—	6·4

When a comparison is made between pairs of similar soils in the same area whose state of fertility is known, it is found that the actual amounts of carbon and nitrogen oxidised by 3 per cent. peroxide are significantly higher in the more fertile than in the less fertile soils. Not only are the absolute amounts of total carbon and oxidisable carbon greater in the fertile soils, but the percentage of oxidisable carbon calculated on the total carbon is also greater.

COMPARISON OF CARBONATE AND CARBONATE-FREE SOILS.

The percentages of total carbon and total nitrogen oxidised by 3 per cent. peroxide in carbonate and carbonate-free soils are given in Table VI.

The percentage of total carbon and total nitrogen oxidised by 3 per cent. peroxide is considerably lower in the carbonate than in the carbonate-free soils. The abnormal behaviour of the Trinidad, Transvaal,

and Kenya soils will be noted. The British carbonate-free soils (eleven in all) give an average figure for the percentage of total carbon oxidised of $85 \cdot 1 \pm 0 \cdot 7$ and for the nitrogen oxidised of $83 \cdot 1 \pm 1 \cdot 0$. The average C/N ratio of the "oxidisable organic matter" of these soils works out at $10 \cdot 0 \pm 0 \cdot 2 : 1$.

Table VI. *Percentages of total carbon and nitrogen oxidised by 3 per cent. peroxide in carbonate and carbonate-free soils.*

Carbonate soils.

	Total		Oxidised by 3 % peroxide					
					Percentage of total			
Soil	C (%)	N (%)	C (%)	N (%)	C	N	CaCO₃ (%)	pH
Broadbalk, F.Y.M. plot, Rothamsted	2·70	0·26	2·091	0·219	77·8	84·3	3·6	—
Broadbalk, no manure plot, Rothamsted	1·06	0·12	0·585	0·076	55·2	63·4	4·1	—
Barnfield, F.Y.M. plot, Rothamsted	2·85	0·28	2·237	0·227	78·5	81·2	2·2	—
Barnfield, no manure plot, Rothamsted	0·71	0·11	0·270	0·046	38·0	41·9	3·0	—
Herefordshire, 139	1·62	0·19	1·301	0·144	80·3	75·8	0·35	—
Herefordshire, 331	1·32	0·15	0·870	0·099	65·9	66·0	0·8	—
Herefordshire, 92	2·42	0·23	2·050	0·182	84·7	79·2	0·1	—
Llysfasi A, Denbighshire	2·33	0·24	1·752	0·193	75·2	80·5	3·0	—
Black soil, Kenya	2·18	0·14	0·552	0·043	25·3	30·6	6·0	—
Black clay, Trinidad	2·16	0·21	1·162	0·115	53·8	54·8	0·25	—
Black turf soil, Transvaal	1·41	0·10	0·623	0·048	44·2	48·0	2·3	—
Carbonate-free soils.								
Stackyard, F.Y.M. plot, Woburn	1·51	0·15	1·290	0·128	85·4	85·3	—	6·2
Stackyard, no manure plot, Woburn	0·90	0·10	0·780	0·075	86·7	75·0	—	5·9
Herefordshire, 26	1·71	0·19	1·373	0·146	80·3	77·0	—	6·4
Aber, T 12	3·26	0·39	2·683	0·328	82·3	84·1	—	5·7
Aber, 2	3·97	0·40	3·540	0·344	89·2	86·0	—	6·1
Aber, 6	2·78	0·29	2·338	0·228	84·1	78·7	—	6·2
Aber, B 12	2·71	0·31	2·257	0·255	83·3	82·3	—	6·0
Madryn 2, Caernarvonshire	4·31	0·39	3·819	0·346	88·6	88·6	—	5·5
Bodrwyn, Anglesey	5·07	0·51	4·593	0·449	90·6	88·1	—	5·5
Bangor	6·80	0·59	5·576	0·522	82·0	88·5	—	5·2
Lledwigan, Anglesey	4·08	0·43	3·390	0·344	83·1	80·0	—	6·5
Padi soil, Siam, B.A.S. 9	6·28	0·54	5·817	0·434	92·6	80·4	—	4·0
Padi soil, Siam, B.A.S. 3	1·63	0·18	1·441	0·113	88·4	62·8	—	4·5
Red clay, Trinidad	1·33	0·25	1·137	0·112	85·5	44·6	—	7·0
Red loam soil, Transvaal	1·87	0·13	1·302	0·086	69·6	66·2	—	7·0

It has already been pointed out that a volume of 60 c.c. of 3 per cent. peroxide for about $0 \cdot 1$ g. organic carbon was sufficient to decompose the "oxidisable organic matter" in carbonate-free soils, but it would seem from the above table that this may not be true for carbonate soils. Accordingly several soils containing varying percentages of calcium carbonate were treated initially with 60 c.c. and with subsequent additions of 40 c.c. of 3 per cent. peroxide. The results for these soils are given in Table VII.

A volume of 60 c.c. of 3 per cent. peroxide is evidently not sufficient to decompose completely the "oxidisable organic matter" of carbonate soils. Not only is more carbon oxidised by further additions of peroxide but more nitrogen is oxidised. *The nitrogen figures would have been constant*

if the original volume of 60 c.c. had been sufficient. The writer is unable to give an explanation of the difference in behaviour of the organic matter of carbonate and carbonate-free soils. The use of a peroxide stronger than 3 per cent. would appear to be indicated for these soils. On referring to Table IV it will be seen that there is no attack even with 5 per cent. peroxide of cellulose added to the Llysfasi, Broadbalk and Herefordshire soils.

Table VII. *Effect of varying volumes of 3 per cent. peroxide on carbonate soils.*

Volume of 3 % peroxide (c.c.)	Black soil, Kenya. Percentages of total C and N oxidised		Black clay, Trinidad. Percentages of total C and N oxidised		Black turf soil, Transvaal. Percentages of total C and N oxidised	
	C	N	C	N	C	N
60	25·3	30·6	53·8	54·8	44·2	48·0
100	43·1	61·2	70·4	65·9	67·5	69·0
140	52·3	83·4	78·3	65·9	74·6	75·0
180	61·0	92·4	82·5	69·8	80·3	77·0

Black soil, Kenya, contains 2·18 % C, 0·14 % N, 6·0 % $CaCO_3$
Black clay, Trinidad, contains 2·16 % C, 0·21 % N, 0·25 % $CaCO_3$
Black turf soil, Transvaal, contains 1·42 % C, 0·10 % N, 2·3 % $CaCO_3$

GENERAL DISCUSSION.

The use of 6 per cent. peroxide which has been proposed for the fractionation of the organic matter of soils has been questioned on the ground that nothing is known of the nature of the organic complexes that are acted on by this reagent. This criticism is undoubtedly justified, as the writer has shown that treatment of a soil with 6 per cent. peroxide removes varying quantities of organic matter, this amount depending not only on the volume of peroxide but also on the quantity of organic matter used in a determination. Furthermore, it has been shown that although 6 per cent. peroxide may not attack cellulose by itself under certain prescribed conditions, it certainly does decompose cellulose in the presence of soil, and this decomposition varies from soil to soil. The constituent or constituents of a soil that are instrumental in bringing about this decomposition are not known. The object in view in treating a soil with peroxide, apart from its use in dispersion for mechanical analysis, is to determine the so-called "degree of humification" of the soil and, if possible, ascertain its relation to state of fertility, etc. The use of 6 per cent. peroxide would, therefore, seem to be unsuitable for the end in view.

A very striking piece of information, however, was obtained by treating soils with 6 per cent. peroxide until frothing ceased and after-

wards continuing the treatment by adding more peroxide in small quantities at a time and boiling between each addition. *Whilst the initial volume of peroxide removed organic compounds containing both carbon and nitrogen, all subsequent additions of peroxide resulted in the oxidation of non-nitrogenous carbon compounds only.* This indicated that in the initial treatment of a soil with 6 *per cent. peroxide,* oxidation of both classes of organic compounds might have occurred.

This was actually found to be the case with most soils, and consequently a search was made for *that particular quantity and concentration of peroxide that would oxidise the organic compounds containing both carbon and nitrogen* and leave the non-nitrogenous carbon compounds unacted upon. Three per cent. peroxide (2 per cent. or even weaker may be found suitable in some instances) was found to be suitable for most non-carbonate soils with a pH of 7 or under and 5 per cent. peroxide for soils containing calcium carbonate. The latter group of soils, however, needs further investigation.

It is therefore suggested that the carbon and nitrogen removed simultaneously by 3 per cent. peroxide must be present in the soil in the form of a definite organic complex. This is not a protein compound as its carbon-nitrogen ratio is approximately $10:1$, whereas the C/N ratio of protein is approximately $3 \cdot 3:1$. It is probable that this complex is a protein compound in intimate association or combined with a complex of carbohydrate character of high carbon content. The writer hopes to bring forward evidence as to the nature of this complex in a future contribution. The carbon of the organic matter of soils that survives this particular treatment is oxidisable—in part at any rate—by more stringent treatment with peroxide, and is most likely to be present in the soil as a non-nitrogenous carbon compound, probably of the nature of cellulose, since the concentration of peroxide in question does not attack this compound. The nitrogen remaining in the residue resulting from the 3 per cent. peroxide treatment is found to be unacted on even by 6 per cent. peroxide in repeated applications at this concentration. There is no doubt whatever that this category of nitrogen is essentially different from the nitrogen that is removed by a single treatment of a very dilute peroxide. It is proposed to designate associated and simultaneously oxidisable nitrogenous and nitrogen-free organic matter as "oxidisable organic matter" and the organic matter that survives the peroxide treatment as "residual organic matter." The carbon and nitrogen in the "oxidisable organic matter" of the British carbonate-free soils examined form from about 85 and 83 per cent. respectively of the carbon

and nitrogen in the total organic matter, and it has been shown that the higher the state of fertility the higher is its content in oxidisable organic carbon and nitrogen.

SUMMARY.

1. Six per cent. peroxide is found to be unsuitable as a reagent for the determination of the "degree of humification" of soil organic matter, as it oxidises not only compounds containing carbon and nitrogen but also a varying amount of a substance containing carbon but no nitrogen, this amount depending on the volume of reagent used and the total amount of carbon originally present in the quantity of soil employed.

2. There are apparently two phases in the attack of peroxide on soil organic matter. In the first phase, material containing carbon and nitrogen is oxidised, whilst in the second phase the material oxidised consists solely of nitrogen-free carbon compounds.

3. By the use of 3 per cent. peroxide (2 per cent. or less may be preferable for some soils) the attack on soil organic matter can be restricted to the first phase. The material thus oxidised appears to be built up by the association of a protein complex with a carbohydrate complex of high carbon content. Its carbon-nitrogen ratio is about 10:1.

4. The residual organic matter surviving treatment with 3 per cent. peroxide consists of nitrogenous compounds unoxidisable even by repeated treatment with 6 per cent. peroxide, and nitrogen-free compounds possibly of a cellulose character which can be almost completely oxidised by treatment with 6 per cent. peroxide.

5. The carbon and nitrogen in the "oxidisable organic matter" form about 85 and 83 per cent. respectively of the total carbon and nitrogen in average mineral carbonate-free soils. Lower figures are obtained for carbonate soils but may be increased by repeated treatment with 3 per cent. peroxide or by the use of stronger peroxide.

6. Comparing similar soils of high and low fertility, it is found that the more fertile soils contain higher percentages of "oxidisable organic matter" than the less fertile.

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THE EFFECTS OF VARIED DRESSINGS OF GROUND LIMESTONE IN THE FIELD.

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(With Three Text-figures.)

In spite of the lack of field experimental data, and of its many weaknesses and limitations, the "lime requirement" estimation of Hutchinson and MacLennan is still largely used by advisory workers and field experimenters.

The two workers named (1), with a Craibstone soil of L.R. 0·42 per cent., got full value in the reduction of this figure with dressings up to the value corresponding to L.R. 0·36 per cent., but above this point they found that the results fell away steadily. Wild (2), examining limed and unlimed soils in New Zealand, found that the reduction in the L.R. figure was always less than the amount of the dressings applied, and so argued that the L.R. figure was consistently higher than the amount of calcium carbonate really necessary. Fisher (3), reviewing the subject of soil reaction generally, stressed the same point and demonstrated several weaknesses of the L.R. estimation, especially in its behaviour with different types of soils. Crowther and Martin (4), in a later publication, produced evidence that the method actually under-estimated the amount of lime necessary to produce neutral soils in practice, and that it could be usefully employed in field work in conjunction with pH determinations. Ogg and Dow (5) have compared these two methods on an extensive series of samples of soil in Scotland.

The following observations made in the course of some English field trials are detailed as providing additional data on these points. The experiments were originally laid down as part of a scheme which included in its objects the perfection of a means of determining the minimum quantity of lime necessary to remove acidity from a soil, and the accumulation of data correlating field results with analytical practice. The work was done on ¼-acre plots, duplicated as far as possible, and receiving six different amounts of ground limestone, from 0 to 60 cwt. per acre. The limestone was from one source, contained 93–95 per cent. calcium carbonate, and was ground until 65–70 per cent. passed the 100-mesh sieve. Before liming and at the end of each subsequent year

the plots were sampled to a depth of 9 in., at least twelve cores per plot being taken.

The types of soil and cropping systems were as follows:

Table I.

Place	Plateau gravel (Wasing, Berks.)	Lower greensand (Nuneham Courtenay, Oxford)	Loam on London clay (Verwood, Dorset)	Plateau gravel (Lee-on-Solent, Hants.)
Sand (%)	66·62	58·60	58·63	51·23
Coarse silt	11·79	16·29	16·87	21·15
Fine silt	6·91	7·56	10·70	10·60
Clay	6·78	6·82	4·65	8·48
Moisture	1·77	3·15	1·09	2·12
Loss on ignition	4·22	4·97	6·16	5·30
$CaCO_3$	0	0	0	0·01
L.R. (as $CaCO_3$)	0·110	0·115	0·092	0·051
Exchangeable CaO	0·115	0·200	0·212	0·230
1924	Swedes	Oats and barley	Swedes	Oats
1925	Barley	Seeds	Oats	Seeds
1926	Seeds	Wheat	Seeds	Wheat

The L.R. figures for a typical centre are given in *Agricultural Progress*, Vol. v(6), and with those for two other centres are set out graphically in Fig. 1, which shows the range of plot variation on any one field, the very uneven effects by the end of the first year after liming, and the regular graded effects at the end of the second and subsequent years.

The Lee-on-Solent centre provided an instance in which the effect of the liming was delayed until the third season through the application of limestone being ploughed in.

Table II. *Original L.R. 0·051 per cent.*

Plot no. ...	1	2	3	4	5	6
Limestone applied (cwt. per acre) ...	0	10	20	30	40	50
L.R. 1924 (%)	0·040	0·050	0·050	0·047	0·040	0·030
L.R. 1925 (%)	0·053	0·043	0·055	0·055	0·050	0·033
L.R. 1926 (%)	0·050	0·030	0·040	0·033	0·016	0·001

As these figures show, it lay undisturbed beneath a seeds ley until late 1925 and did not become evenly disseminated through the soil, so as to exert its full effect, until then.

In every case the reduction of the L.R. figure is only a fraction of the amount of limestone added, but it seems to be persistent for several years and to be of the same order in the three examples plotted.

The amounts of calcium carbonate found on the plots in successive years were estimated by means of Collin's calcimeter and are set out for two centres in Fig. 2.

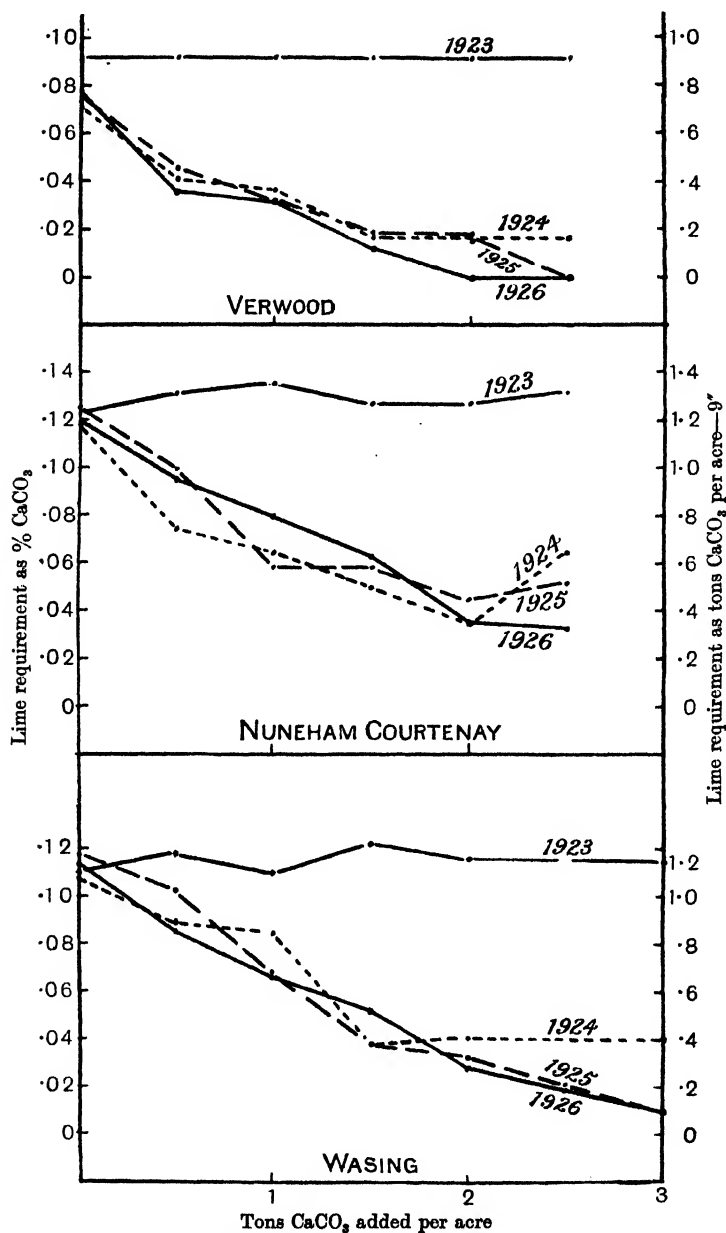In an effort to trace the fate of the dressings put on to these plots,

Fig. 1. Shewing lime requirement in successive years.

the exchangeable and total CaO were determined as far as possible. The conditions at Wasing at the end of the third year are set out in Fig. 3, which clearly shows the increase in these two figures which is effected, and the approach to the saturation point. There was still, on the plots
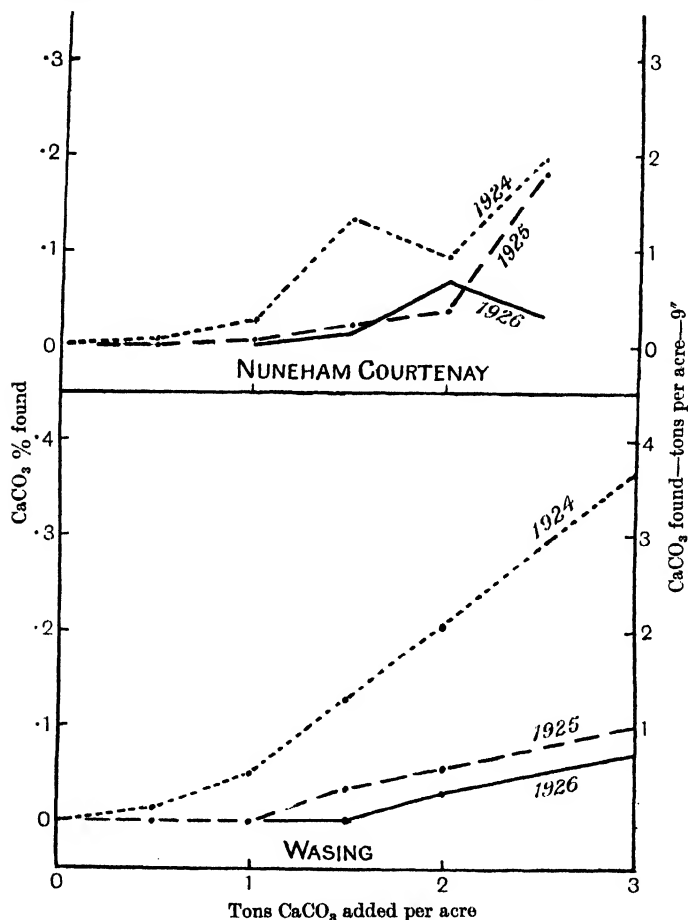
Fig. 2. Showing $CaCO_3$ found in successive years (limestone applied in 1923).

receiving 2 and 3 tons of ground limestone, a small residue of these dressings. An examination of the results shows the rapidity with which the calcium is adsorbed and the extent to which this action goes. It would appear that, in the period of the experiment, this was the main effect and that losses by leaching and drainage are not considerable. Page and Williams (7) investigated this point by storing treated soils in

a damp condition. They found that up to a certain point all the calcium carbonate added could be found in the increased exchangeable calcium base present.
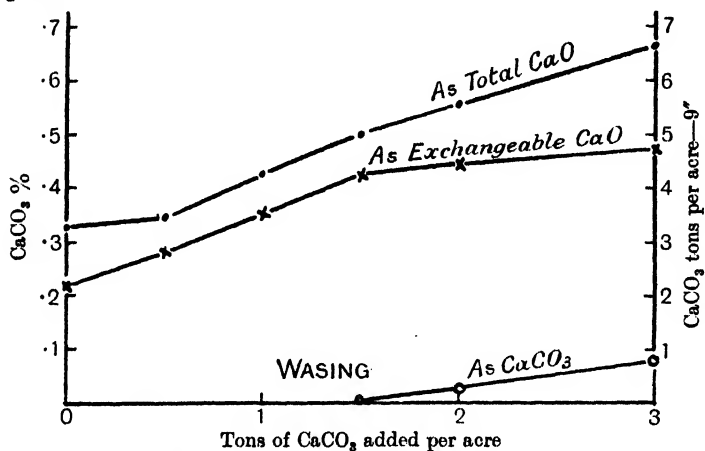

Fig. 3. Showing lime status of plots 3 years after addition of $CaCO_3$.

SUMMARY.

1. These field experiments show that equilibrium after applications of lime may be attained on arable land between one and two years later.

2. The quantitative correcting effects of a given dressing seem to be about equal on different soils.

3. Added calcium carbonate is quickly converted into exchangeable calcium base to an extent which depends on the individual soil.

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NUTRITIVE VALUE OF PASTURE.

VII. THE INFLUENCE OF THE INTENSITY OF GRAZING ON THE YIELD, COMPOSITION AND NUTRITIVE VALUE OF PASTURE HERBAGE (PART III).

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INTRODUCTION.

In previous publications the results of investigations into the yield, composition and nutritive value of pasturage under systems of weekly (1, 2), fortnightly (3) and 3-weekly (4) cuts have been brought forward. It has been shown that so far as digestibility and nutritive value (expressed in terms of starch equivalent) are concerned, there is little to distinguish grass cut at weekly, fortnightly and 3-weekly intervals. Under a system of 3-weekly cuts, pasture grass never reaches the stage of growth at which lignification sets in, with consequent decline in the digestibility of not only the fibrous constituent, but also of the other organic ingredients. It still retains the non-lignified, highly digestible character of grass cut at weekly or at fortnightly intervals, and since this characteristic of high digestibility is maintained, by cutting every 3 weeks, over the entire season, it may be inferred that a similar result would follow from a system of rotational grazing, where the pasture enclosures, after being closely grazed, are permitted a 3-weeks' interval of unchecked growth before being grazed again.

It has further been demonstrated that grass cut at 3-weekly intervals contains rather less crude protein, and slightly more crude fibre and N-free extractives, than grass grown under systems of weekly and fortnightly cutting. On the basis of dry matter, it has been found that the average crude protein content of weekly cut grass is about 25 per cent., that of fortnightly cut grass about 23·5 per cent. and that of 3-weekly cut grass about 21 per cent. The depressing influence of drought on the digestibility of grass, and its content of crude protein, has been shown to be less pronounced under a 3-weekly system of cuts than under systems of more frequent cutting.

Although the nutritive ratio of the herbage from the experimental

pasture is gradually becoming wider as the system of cutting becomes less severe, it was found that the values for grass cut every 3 weeks were still significantly narrower than that for milk not only during the early part of the 1928 season, but also during July, August and September. For these periods of the season, therefore, the conclusion still held good respecting the desirability of employing carbohydrate-rich foods for the supplementary feeding of pasturing animals. During May and June 1928 (3-weekly cutting), however, the nutritive ratio of the pasturage widened to a value almost equal to that of milk. At this stage, the grass was adapted to the requirements of young stock and was approaching a balance suitable for dairy cows yielding from 3 to 5 gallons of milk per day. For fattening stock, however, a supplement of carbohydrate-rich food would still have been necessary in order to furnish such animals with a diet balanced according to the accepted feeding standards.

In respect of the yield of nutrient matter from the pasture under different systems of cutting, it has been found that this increases progressively as the interval between successive cuttings is lengthened from a week to a fortnight, and from a fortnight to 3 weeks. Further, the disparities in productivity have been shown to be most outstanding at those periods of the season when unfavourable meteorological conditions for growth were prevalent. It was demonstrated that if the management of the experimental pasture had been attempted along the lines of close-grazing *under the weather conditions of* 1928, a simple division of the main field into a suitable number of smaller enclosures, for close-grazing at 3-weekly intervals, would have enabled the stock-carrying capacity of such *unfertilised* pasture to be increased in the ratio of 2:3.

THE PRESENT INVESTIGATIONS (SEASONS 1929 AND 1930).

The pasture trials to be described in the present communication were carried out during the seasons of 1929 and 1930 and were designed to secure data respecting the yield, composition and nutritive value of pasturage under a system in which the interval between successive cuttings had been lengthened to a month. The previous investigations have indicated that the question of the maximum yield of starch equivalent from pastures is bound up with the investigation of the process of lignification in the herbage, since the conditions for such maximum yield will presumably be realised when the interval between successive cuttings, or grazings, is as long as possible. The length of this optimum interval will in turn be conditioned by the time required for the young

shoots of grass to reach the stage of growth at which lignification sets in. The results of the present trials may be taken as conforming with those which would be realised under a monthly rotational close-grazing system. They are therefore invested with special significance, in that a period of four weeks has been tentatively adopted in this country as the interval which is allowed to lapse, in rotational grazing practice, between successive grazings of pasture enclosures.

First investigation (1929 season).

The experiments were carried out on the light-land pasture on which the 1925 (weekly cutting)(1), the 1927 (fortnightly cutting)(3) and the 1928 (3-weekly cutting)(4) trials had been conducted. As in past years, the results for the 1929 season refer to pasturage under unfertilised conditions. Apart from a dressing of basic slag, which was applied at the rate of 10 cwt. to the acre in the autumn of 1925, the pasture has not received artificial fertilisers during the past decade. During the winters between the investigations, the pasture plot has been lightly grazed by the ewe flock and in this way has been enriched, to a moderate extent, in respect of organic matter.

The main pasture plot was divided into thirty equal sub-plots, each measuring 210 by 18¼ links. Sub-plot 10 was reserved for cutting every week, a treatment to which the area comprising this sub-plot had also been subjected during the previous season. The remaining sub-plots were cut, by means of a motor lawn mower, on successive days throughout the season. Since sub-plots 11 and 12 were cut together as one daily sub-plot (for reasons which concern the next set of trials carried out during the season of 1930), it follows that the whole area of the main plot was cut over once in every month. After the cutting of a daily sub-plot, the herbage was weighed, mixed thoroughly and sampled for chemical analysis. A suitable bulk was then abstracted for feeding to sheep on which digestion trials were being carried out. In addition to the pasture sub-plots, the usual contiguous area was reserved, as in past years, for the purpose of hay and aftermath trials.

Towards the end of February 1929 the pasture plot was lightly rolled, and the lawn mower was taken over the whole area in the early part of March. Systematic cutting of the sub-plots was begun on March 15, so that by April 12 the herbage secured day by day represented the produce of a month's unchecked growth. Daily cutting of the sub-plots was continued throughout the season until the conclusion of the trial on October 3.

Second Investigation (1930 *season*).

The severe and prolonged drought of the 1929 season (see Table I) exerted an overwhelming influence on the productivity of the pasture and on the composition and feeding value of the herbage, and it was evident that the results could not permit of decisive conclusions respecting the question under investigation, namely, the influence of widening to a month the interval between successive cuttings. For that reason, it was decided to repeat the work during the season of 1930. Since signs had not been lacking during the 1929 season that the pasture was beginning to suffer from soil impoverishment, it was deemed advisable to carry out the 1930 work under conditions of intensive fertilising.

On November 11, 1929, well-rotted farm yard manure was applied, by means of a mechanical distributor, to all the sub-plots, including the hay plot, at the rate of 5 tons to the acre. From December 13 to 18, 1929, the following dressing was distributed by hand over the pasture sub-plots: ground chalk, 2 tons per acre; superphosphate of lime, 5 cwt. per acre; sulphate of potash, 2 cwt. per acre. Special precautions were taken to ensure uniform distribution. The mixture was not applied, however, to sub-plot 10 (weekly unmanured control sub-plot) and to sub-plot 11 (monthly unmanured control sub-plot).

The hay plot, measuring $\frac{1}{20}$ acre, was divided into three equal strips (meadow sub-plots 1, 2 and 3), and the above mixture of fertilisers was applied to the two outside strips, but not to the middle strip.

During the first week of January 1930 the plot was vigorously raked over with wooden rakes to spread and move the farm yard manure, such treatment having the beneficial effect associated with harrowing. On February 17 and 18, all the pasture sub-plots, with the exception of sub-plots 10 and 11, were dressed with neutral sulphate of ammonia at the rate of $\frac{5}{8}$ cwt. per acre. At this stage, the herbage was verdant and in very good condition, the ground chalk, etc. applied in the previous December having already been well washed into the soil. Sulphate of ammonia was also applied to meadow sub-plot 3 at the rate of 2 cwt. per acre.

From March 3 to 7 the whole plot was cut over to remove winter growth, and the small amount of fibrous material remaining from the application of the farm yard manure was raked off. The preliminary day-by-day cutting of the pasture sub-plots was begun on March 15, so that by April 12 the herbage secured in this manner represented the

produce of a month's unchecked growth. After every cutting of a daily sub-plot, sulphate of ammonia was applied at the rate of $\frac{3}{8}$ cwt. per acre. The daily cuttings, followed by application of sulphate of ammonia, were continued until the conclusion of the trials on October 3. It should be pointed out, however, that the dressings of sulphate of ammonia were omitted in the months commencing June 7 and August 30, and that the control sub-plots 10 and 11, apart from the dressing of farm yard manure in the autumn of 1929, received no fertilisers of any kind throughout the whole trial.

Table I. *General meteorological conditions during the seasons* 1929 *and* 1930.

Month ...	Apr.		May		June		July		Aug.		Sept.	
Year ...	1929	1930	1929	1930	1929	1930	1929	1930	1929	1930	1929	1930
Rainfall in inches	1·25	2·36	2·81	3·05	0·81	1·70	2·19	2·82	0·40	1·66	0·12	3·00
Average per day	0·042	0·079	0·090	0·099	0·027	0·057	0·070	0·091	0·013	0·054	0·004	0·100
No. of rainy days (0·01 in. or over)	13	12	9	16	9	4	11	10	8	12	3	13
Sunshine in hrs.	148·3	111·0	248·6	170·3	204·1	239·8	235·3	182·8	184·9	237·2	190·0	129·1
Average per day	4·9	3·7	8·0	5·5	6·8	8·0	7·6	5·9	6·0	7·7	6·3	4·3
No. of sunless days (1 hr. or less)	6	13	4	6	3	3	4	5	2	1	3	8
No. of absolutely sunless days	4	5	2	1	1	1	1	3	0	1	1	8
Mean max. daily temp. (° F.)	53·4	54·1	62·9	60·0	66·6	69·9	72·7	70·0	71·8	71·0	71·0	65·1
Mean min. night temp. (° F.)	35·3	40·3	41·7	43·8	46·8	50·2	50·7	53·1	51·1	53·2	51·0	50·3

	1929 in.	1930 in.
Total rainfall in January	1·25	1·53
,, February	0·50	0·58
,, March	0·03	1·58

Comments on Table I.

The seasons of 1929 and 1930 were sharply contrasted in respect of weather, 1929 being a season of abnormally low rainfall, whereas 1930 was characterised by an abundance of rain, which was fairly well distributed over the entire season. The total rainfall for 1929 (April to September, inclusive) was only 7·58 in., compared with 10·1, 13·1, 13·2 and 10·0 in. over the corresponding seasons of 1928, 1927, 1926 and 1925 respectively. The total rainfall for the same period of 1930 was 14·59 in., that is to say, the highest recorded since the beginning of this series of investigations.

The winter prior to the 1929 investigation had been unusually cold and dry. The four months January to April were the driest in the London area since regular weather records began to be kept in 1815.

Over the country as a whole, March, with its total rainfall of but 0·03 in., was the driest March experienced since 1870. It was evident at this early stage of the season that growth on pastures would be seriously affected and that the hay crop would probably be late and thin. The commencement of active growth on the pasture plot was further retarded by a succession of cold days in late April and early May, and the prevalence of ground frosts at night. Towards the end of the first half of May, rain fell in adequate amount. This circumstance, combined with a rise in the day temperatures, caused the pasture to display signs of awakened activity. May 15 to 23 was rainless, but growth was greatly stimulated by a heavy thunderstorm on May 24, at which date the daily temperatures rose considerably. The "zenith" period of growth, however, was delayed until the end of May, the biggest yields from the sub-plots being recorded over the period from May 29 to June 8.

The circumstances attending the early period of the 1930 investigation were very different from the foregoing. The previous winter up to the end of January had been characterised by mild temperature and heavy rainfall. Although February was moderately cold and dry, the succeeding month had a much more abundant rainfall than was recorded in the droughty March of 1929. By the end of March, fresh growth was already noticeable on the sub-plots and the herbage had a good appearance. April was both wetter and milder than the corresponding period in 1929, while the rainfall in May was also very copious. The biggest yields from the sub-plots were obtained over the period May 17 to 31, some ten days or so earlier than was the case in the 1929 season. That this earlier "zenith" period was to be attributed to the more favourable weather conditions of 1930, and not to the treatment of the plot with fertilisers, is evident from the fact that this feature was shared by manured and unmanured sub-plots alike.

The second half of the 1929 season was further characterised by droughty conditions which were entirely unfavourable to the growth of herbage. Only 0·8 in. of rain fell in June. Although severe thunderstorms brought the July rainfall up to a moderate total, the prominent feature of this month's weather was the persistence of droughty conditions. Of the month's total of 2·19 in. of rain, 1·66 in. fell on 3 days (July 3, 4 and 20). July opened with chilly weather. On July 9, however, a warm spell set in, and this developed into a heat wave of a fortnight's duration, daily temperatures in the neighbourhood of 80° F. being recorded. The pasture suffered severely in this period of drought, heat and excessive sunshine, and the rations of the sheep contained a considerable

proportion of brown, parched herbage (period 4). Conditions of drought and heat prevailed right through August and September, 1929. Apart from 0·02 in. of rain on September 21, the period from August 23 to September 28 was one of unbroken drought. During this time, the pasture presented a bare and brown appearance, with glimpses of green here and there in the furrows. No digestion trial with the sheep was possible during September owing to the almost complete cessation of growth on the pasture plot.

The main justification for publishing the 1929 results is that they give a continuous record of the influence of long-continued drought on the yield, composition and feeding value of the herbage from a pasture, the productive capacity of which has been the subject of detailed study in years of more normal weather conditions.

In sharp contrast with the foregoing, the weather conditions of the second half of the 1930 season could scarcely have been more favourable to the abundant growth of herbage on pastures. Although June, on the whole, was a dry, warm and sunny month, a severe thunderstorm (1·58 in. of rain) on June 18 prevented the pasture from suffering too severely from lack of moisture. The period from June 24 to July 13 was rainless, but despite the dry weather, the herbage remained fresh and green, although the yield was falling steadily. In the latter half of July, 2·82 in. of rain fell. The unsettled weather continued through the greater part of August, although, at the end of this month, a heat wave of great intensity was encountered. This, however, was of short duration and did not affect the pasture adversely. Good growing conditions continued to prevail until the completion of the trial on October 3, at which stage the pasture presented a fresh, verdant appearance more characteristic of spring than of autumn. It will be noted, therefore, that the meteorological conditions of the 1930 season were, on the whole, entirely favourable to the reliable elucidation of the questions under investigation.

BOTANICAL NOTES FOR SEASONS 1929 AND 1930.

As in previous years, the writers had the advantage of the co-operation of their colleague, Mr S. F. Armstrong, M.A., in connection with the botanical phases of the investigations. Periodic inspections of the sub-plots were made with a view to determining the trend of the botanical changes and for the purpose of noting the stages of growth to which the main forms of herbage attained at the different stages of the season.

First investigation (1929 *season*).

Digestion period 1 (*April* 20 *to* 29, 1929). At this stage of the season, creeping bent and perennial rye grass were the most prominent grasses in the pasture, and together constituted about 40 per cent. of the mown herbage. Other grasses, in order of abundance, were rough stalked meadow grass, red fescue and cocksfoot, with small amounts of timothy, meadow foxtail, crested dogstail, Yorkshire fog, soft Brome grass, yellow oat grass and sweet vernal grass. Wild white clover was fairly abundant in patches. Yarrow and burnet were also present in plentiful amount, whilst the prominent weeds were dandelions, buttercups and daisies.

The herbage, with the exception of a small amount of flowering daisies, consisted entirely of leafy growth, 2 to 3 in. in height. Numerous darker green patches of herbage, 4 to 5 in. in height, marked the effect of the droppings of sheep during the period of grazing in the previous winter. The general appearance of the plot suggested that the pasture might be suffering not only from insufficient moisture, but also from lack of ample supplies of plant food material.

Digestion period 2 (*May* 14 *to* 27, 1929). Night frosts were encountered in the early days of May, which caused the longest blades of grass to appear nipped and tipped with brown. Creeping bent, perennial rye grass and red fescue were the most abundant grasses. The grasses constituted about 50 per cent. of the mown herbage, yarrow and burnet together about 10 per cent., wild white clover and red clover about 10 per cent. and weeds in the neighbourhood of 30 per cent. The species were present in the following forms: *leafage only*—creeping bent, Yorkshire fog, meadow fescue and wild white clover; *flower stems emerging*—perennial rye grass, rough stalked meadow grass, crested dogstail, red fescue, yarrow and burnet; *flowering stage*—meadow foxtail, sweet vernal grass, buttercups, daisies, dandelions and plantains.

The pasture appeared to lack good "bottom" growth. The general height of the grass was 2 to 4 in., with stems 6 to 8 in. The proportion of stems increased very considerably in the final stages of this period and it became necessary to follow the mower to pick by hand any stems which escaped the action of the cutter.

Digestion period 3 (*June* 8 *to* 18, 1929). During this period, creeping bent, the predominant grass, reached the early flowering stage. The following species had also attained the flowering stage: perennial rye grass, rough stalked meadow grass, crested dogstail, Yorkshire fog, red fescue and yellow oat grass. Yarrow and burnet were flowering and a

few flowers of wild white and red clover were also in evidence. The herbage during this period was thin and stemmy, lacking density close to the ground.

Digestion period 4 (*July* 13 *to* 26, 1929). The effects of the dry June and the hot, dry days of mid-July were manifest during this period. At the beginning, the herbage was "scorched" over large parts of the plot. The grasses, which were up to 3 in. in height in the best patches, consisted almost exclusively of leafage. The drought-resisting species, such as wild white clover (flowering), yarrow and burnet, were well in evidence, and flowering hawkweeds and plantains were plentiful. As the period advanced, the sward recovered somewhat its normal green appearance, the recovery being assisted by a heavy thunderstorm on July 20. Creeping bent, perennial rye grass, Yorkshire fog and red fescue, all represented by short leafy growth, were the most abundant grasses. On several days during this period, the sub-plots did not yield sufficient herbage to enable the full ration to be fed to the sheep. The herbage contained a considerable proportion of brown, dead grass and its dry matter content was very high (see Table II).

Digestion period 5 (*August* 11 *to* 24, 1929). The effects of continuous heat and drought were intensified during this period. The pasture was brown and parched. Of the grasses, only cocksfoot and red fescue presented a fresh appearance. Creeping bent suffered especially as a consequence of lack of moisture, and even wild white clover was no longer able to thrive. Yarrow and burnet, however, were still holding out against the drought. The herbage throughout the period was very short and leafy, the proportion of brown, dried-up material being very high. On one day only was sufficient herbage obtained to enable the full ration of 4000 gm. to be fed to the sheep. To a large extent, however, this was compensated by the high dry matter content of the herbage.

September period, 1929. During the extension of the drought throughout this month, the pasture was bare and brown, with isolated small green patches in the furrows. Growth had ceased almost entirely, and only sufficient herbage for the purpose of chemical analysis was secured by the mowing machine. This consisted mainly of dried-up creeping bent and red fescue. No digestion trial was carried out during this final month.

Second investigation (1930 *season*).

Digestion period 1 (*April* 15 *to* 28, 1930). By the third week in April, the herbage was growing well and was of a good colour. It varied from 2 to 4½ in. in height and was of a very mixed character in respect of

botanical composition. The grasses, represented almost entirely by foliage, were estimated to form more than 60 per cent. of the mown herbage, the order of abundance being creeping bent, perennial rye grass (which attained a height of 6 to 8 in. by the end of the period) and red fescue. Rough stalked meadow grass, yellow oat grass and Yorkshire fog were also common. Among the species more sparsely represented, sweet vernal grass had reached the stage of flowering.

Yarrow was plentiful, but wild white clover was very scarce. Flower buds were forming on the small amount of burnet present. The weeds, comprising 20 to 25 per cent. of the herbage, included bulbous buttercup (some flower buds formed), plantains, flowering dandelions, daisies, woodrush and lesser celandine. Knapweed, speedwell and chickweed were also present in small amounts.

Digestion period 2 (*May* 10 *to* 23, 1930). The rate of growth of herbage increased very considerably during this period, the general height of the grass being from 3 to 6 in. Towards the end of the period, perennial rye grass had grown to a height of 8 to 9 in., with numerous stems up to 12 in. From May 17 to June 11 it was found necessary to mow the herbage with a scythe, using the lawn mower merely for removing any grass which escaped the action of the scythe. The necessary further precautions were taken in sampling the herbage for analytical and feeding purposes.

The herbage obtained during this period was of very good quality and colour. The grasses predominated and constituted 75 to 80 per cent. of the bulk of the mown herbage. Creeping bent, perennial rye grass and red fescue were again abundant, the rye grass and fescue having reached the early flowering stage towards the end of the period. Other common grasses during this period were sweet vernal grass (flowering), meadow foxtail (flowering), Yorkshire fog (leafage only), yellow oat grass, cocksfoot, rough stalked meadow grass and crested dogstail (in all four cases flower stems emerging).

Yarrow was still common, but formed a smaller proportion of the bulk of the herbage than in April. Burnet was flowering. Wild white clover was scarce. The weeds were relatively less abundant than in the previous month.

Digestion period 3 (*June* 7 *to* 20, 1930). The herbage during this drier month was shorter (2 to 4 in.) at the time of cutting than during the preceding month. The grasses formed about 70 per cent. of the mown herbage. Creeping bent was still the most abundant species, with red fescue taking second place. Rye grass had fallen off appreciably in

amount, while cocksfoot and Yorkshire fog had displayed a slight increase. Yarrow had also increased very definitely, but wild white clover was still as scarce as in the preceding periods. Of the weeds, buttercups had now become comparatively scarce, while plantains, knapweed, dandelions and daisies were still abundant.

Apart from the weeds, few species succeeded in reaching the flowering stage during this period. Many of the grasses were able to produce young flower stems, but none actually flowered. Creeping bent and red fescue, which formed the bulk of the "bottom" growth, contributed leafage only, and the herbage, as a whole, was characterised by leafiness.

Digestion period 4 (*July* 5 *to* 18, 1930). The botanical character of the herbage during July remained much the same as in June. The dry conditions of June and early July, however, reacted disadvantageously on the general vigour of the herbage, which as a consequence became thin and "patchy" in places. Its height varied from 2 to 5 in. Weeds remained abundant, but of these, only plantains were in flower. The latter were excluded from the analytical and feeding samples. The only other species seen in flower were a few plants of creeping bent, wild white and red clover. The bulk of the mown herbage, therefore, consisted of leaves.

Digestion period 5 (*August* 9 *to* 22, 1930). The rains during the latter part of July maintained a freshness of growth on the pasture plot during August. The herbage was short (2 to 3 in.) in the early part of this month, but became longer and more vigorous by mid-August. Creeping bent and red fescue were the most abundant grasses, followed by perennial rye grass, cocksfoot and Yorkshire fog. Yarrow was still abundant, forming as much as 10 per cent. of the yield of herbage. Weed had decreased somewhat in amount, while wild white clover remained very scarce. With the exception of a few plants of plantains, no flowering species were noted, and, as a consequence, the herbage fed to the sheep was essentially leafy in character.

Digestion period 6 (*September* 6 *to* 19, 1930). The wetness of the season maintained a leafy and verdant growth on the pasture throughout this final month, the height of the herbage being from 2 to 4 in. The botanical composition of the herbage remained substantially the same as in August, except that the contribution of yarrow fell away to about 5 per cent. The weeds also gradually diminished in amount, whereas wild white clover appeared to increase slightly. Only one or two weed species flowered to a small extent, and throughout the whole month the mown herbage consisted almost wholly of leaf.

Although the present investigation was not designed primarily to test the influence of intensive fertilising (including the use of sulphate of ammonia) on the botanical character of the herbage of the pasture, it is of interest to compare the observations which were made throughout the season on sub-plot 11 (monthly cut, unfertilised control sub-plot) and sub-plot 10 (weekly cut, unfertilised control sub-plot) with those made on the main plot which was subjected to the system of intensive fertilising. In assessing the significance of these findings, however, it is important to bear in mind that they are based on the results of one year's work only, and for that reason must be regarded as somewhat tentative. Fuller discussion of this question must await the publication of the results of a separate investigation which has been undertaken specifically to test this and kindred points.

The main differences noted in the present investigation are as follows: During the earlier part of the season, the herbage on the monthly cut unfertilised sub-plot 11 was shorter and displayed less vigorous growth than that on the fertilised sub-plots, this difference applying with particular force to the grasses. The herbage also was even more miscellaneous in character, there being less grass and more weeds on sub-plot 11. These differences continued to be displayed throughout the season. In mid-July, following the dry weather of June and early July, sub-plot 11 was browner and barer than the fertilised sub-plots and displayed a slower recovery under the influence of the rains in the second half of the latter month. The proportion of creeping bent was higher in the herbage of this sub-plot, whereas perennial rye grass was not so abundant as on the fertilised part of the pasture plot. At this date, weeds and yarrow were more common, and grasses distinctly less abundant, on the unfertilised sub-plot.

Similar differences were also noted in August and September, sub-plot 11 uniformly exhibiting lack of colour and vigour as compared with the fertilised sub-plots. On the other hand, however, it should be noted that wild white clover developed to a slightly greater extent on the monthly cut unfertilised sub-plot than on the monthly cut fertilised sub-plots, although in this respect sub-plot 11 itself lagged far behind the weekly-cut unfertilised sub-plot 10.

The herbage on sub-plot 10 (weekly cut, unfertilised control sub-plot) was naturally shorter than on the monthly cut sub-plots. Except for a few flowering weeds, the produce of this sub-plot was entirely leafy in character. Owing to the short, leafy character of the herbage and to the presence of more wild white clover, sub-plot 10 always had a darker

green colour than any of the monthly mown sub-plots. Creeping bent, which, on account of its habit of growth, is greatly encouraged and favoured by the action of the lawn mower, was easily the dominant grass and formed quite half the yield during May. Red fescue was also abundant, whereas perennial rye grass, although common in April and early May, appeared to diminish in amount as the season advanced. Wild white clover grew much more strongly on this sub-plot than on any of the others, a feature which was to be attributed to the shortness of the grass in consequence of the more frequent cutting. Yarrow was always abundant on sub-plot 10 and reached its maximum development about midsummer, at which stage it constituted about 20 per cent. of the yield.

SEASONAL VARIATIONS IN THE CHEMICAL COMPOSITION OF THE MONTHLY PASTURE CUTS (1929 AND 1930).

The data in Table II give a continuous record of the composition, on the basis of dry matter, of the monthly cut grass from the commencement of the trials in April to their conclusion in early October. The figures for the various composite samples represent the mean composition of the herbage obtained from the sub-plots over the periods indicated in the date column. As in previous years, the data have been reduced to the basis of soil-free dry matter; that is to say, they have been calculated on the basis of a common silica content of 1·72 per cent. This figure, however, must not be taken as representing the actual silica content of soil-free grass. The reasons for the adoption of this basis of comparison have been stated in the first publication of this series. Throughout the two seasons of investigation, however, contamination of the grass samples with soil was only slight, so that the corrections of the analytical data for soil content were correspondingly insignificant. The figures for the mean moisture content of the herbage as cut during the different periods of the trials are also included in Table II.

Comments on Table II.

The significance of the figures in. Table II will best be appreciated by comparing them with the results which were obtained in 1925, 1927 and 1928 under systems of weekly, fortnightly and 3-weekly cutting respectively. The comparison is shown in Table III, together with the data obtained from the weekly cut sub-plot in 1929 and 1930 and from the unmanured monthly cut control sub-plot in 1930.

Table II. Composition of soil-free samples of pasture grass from light-land pasture under system of monthly cuts during 1929 and 1930 seasons (dry matter basis).

First investigation (Season 1929).

Digestion period		1		2		3		4		5		
Date of cutting (1929)	Apr. 12–Apr. 19	Apr. 20–Apr. 29	Apr. 30–May 13	May 14–May 27	May 28–June 7	June 8–June 18	June 19–July 12	July 13–July 26	July 27–Aug. 10	Aug. 11–Aug. 24	Aug. 25–Sept. 20	Sept. 21–Oct. 3
	%	%	%	%	%	%	%	%	%	%	%	%
Crude protein	25·93	22·66	21·75	18·88	18·71	18·23	18·21	19·11	19·83	19·04	15·46	13·63
Ether extract	7·06	5·67	5·62	7·69	6·64	6·79	5·30	5·92	7·62	6·78	5·29	5·03
N-free extractives	45·28	50·40	49·87	46·26	46·59	47·19	50·48	47·49	44·83	47·36	51·34	52·42
Crude fibre	14·04	13·20	14·61	16·85	18·84	18·96	17·70	18·75	17·74	18·51	19·79	20·10
Ash	7·69	8·07	8·15	10·32	9·22	8·83	8·31	8·73	9·98	8·31	8·12	8·82
True protein	22·82	21·03	19·04	17·21	16·50	16·64	16·59	17·24	17·16	16·17	13·46	12·00
"Amides"	3·11	1·63	1·81	1·67	2·21	1·59	1·62	1·87	2·67	2·87	2·00	1·63
Lime (CaO)	1·31	1·42	1·66	1·60	1·73	1·72	1·80	1·86	1·85	1·73	2·56	2·04
Phosphoric acid (P_2O_5)	0·92	0·89	0·92	0·90	0·94	1·03	1·17	1·06	0·93	0·99	0·82	0·76
Moisture as cut	74·70	74·10	76·20	76·60	79·30	76·00	70·60	65·70	71·20	62·90	55·00	49·70

Second investigation (Season 1930).

Digestion period		1		2		3		4		5		6	
Date of cutting (1930)	Apr. 15–Apr. 28	Apr. 29–May 9	May 10–May 23	May 24–June 6	June 7–June 20	June 21–July 4	July 5–July 18	July 19–Aug. 8	Aug. 9–Aug. 22	Aug. 23–Sept. 5	Sept. 6–Sept. 19	Sept. 20–Oct. 3
	%	%	%	%	%	%	%	%	%	%	%	%
Crude protein	23·52	22·09	17·20	17·30	17·24	19·56	17·37	19·19	18·28	18·79	19·47	22·20
Ether extract	3·43	3·46	4·18	3·63	4·10	4·48	3·91	4·04	4·34	5·11	4·80	4·26
N-free extractives	45·90	46·12	46·13	48·18	45·39	46·45	48·92	46·28	47·00	45·29	46·31	43·21
Crude fibre	17·72	19·11	23·35	21·93	24·29	20·38	20·95	21·88	21·66	21·89	20·26	20·99
Ash	9·43	9·22	9·14	8·96	8·98	9·13	8·85	8·61	8·72	8·92	9·16	9·34
True protein	22·03	19·80	15·33	15·90	15·60	17·94	15·37	17·13	16·29	17·36	18·04	20·03
"Amides"	1·49	2·29	1·87	1·40	1·64	1·62	2·00	2·06	1·99	1·43	1·43	2·17
Lime	1·30	1·23	1·08	1·10	1·16	1·27	1·36	1·39	1·46	1·46	1·40	1·51
Phosphoric acid	1·04	1·08	1·04	1·05	0·98	0·99	0·98	1·11	1·08	1·12	1·15	1·25
Moisture as cut	80·30	82·90	81·80	83·00	78·40	77·80	71·50	78·40	80·30	78·60	78·40	80·00

Table III. *Showing comparison between composition of pasture grass from light-land pasture plot under weekly (1925, 1929 and 1930), fortnightly (1927), 3-weekly (1928) and monthly (1929 and 1930) systems of cutting (dry matter basis).*

Season	1925 (4)			1927 (4)			1928 (4)		
System of cutting	Weekly (without fertilisers)			Fortnightly (without fertilisers)			3-weekly (without fertilisers)		
	Extreme values		Mean value	Extreme values		Mean value	Extreme values		Mean value
	%	%	%	%	%	%	%	%	%
Crude protein	21·20	27·92	24·74	21·31	27·93	23·48	19·29	24·89	21·14
Ether extract	4·72	6·45	5·29	5·76	7·55	6·53	5·48	6·60	6·04
N-free extractives	42·04	52·11	44·79	41·67	47·61	44·53	43·38	48·94	46·68
Crude fibre	12·33	17·68	15·39	12·91	17·23	15·94	14·28	18·82	17·16
SiO_2-free ash	7·26	8·72	7·77	6·88	8·82	7·80	6·88	7·66	7·26
True protein	18·13	23·85	20·95	19·18	25·26	20·84	16·84	21·55	18·66
"Amides"	2·20	5·62	3·79	2·12	3·40	2·64	2·02	3·34	2·48
Lime	1·23	1·81	1·53	1·29	1·68	1·50	1·36	1·63	1·49
Phosphoric acid	0·87	1·14	1·03	1·01	1·29	1·15	1·00	1·20	1·09
Moisture as cut	69·00	80·50	76·00	72·50	79·80	77·20	68·90	80·80	76·30

Season	1929*			1930			1930		
System of cutting	Monthly (without fertilisers)			Monthly (without fertilisers)			Monthly (with fertilisers)		
	Extreme values		Mean value	Extreme values		Mean value	Extreme values		Mean value
	%	%	%	%	%	%	%	%	%
Crude protein	18·21	25·93	20·23	16·08	22·24	19·16	17·20	23·52	19·35
Ether extract	5·30	7·69	6·51	3·09	5·08	3·99	3·43	5·11	4·14
N-free extractives	44·83	50·48	47·58	45·54	50·19	47·79	43·21	48·97	46·27
Crude fibre	13·20	18·96	16·92	16·85	23·09	20·67	17·72	24·29	21·20
SiO_2-free ash	5·97	8·60	7·04	6·13	7·05	6·67	6·89	7·71	7·32
True protein	16·17	22·82	18·13	14·44	19·80	17·09	15·33	22·03	17·57
"Amides"	1·59	3·11	2·10	1·64	2·45	2·07	1·40	2·29	1·78
Lime	1·31	1·86	1·67	1·18	1·64	1·41	1·08	1·51	1·31
Phosphoric acid	0·89	1·17	0·98	0·90	1·36	1·07	0·98	1·25	1·07
Moisture as cut	62·90	79·30	72·70	69·80	81·00	76·20	71·50	83·00	79·30

Season	1929*			1930		
System of cutting	Weekly (without fertilisers)			Weekly (without fertilisers)		
	Extreme values		Mean value	Extreme values		Mean value
	%	%	%	%	%	%
Crude protein	20·89	26·59	23·26	20·04	25·81	23·43
Ether extract	5·24	7·37	6·70	3·75	4·55	4·26
N-free extractives	44·33	49·69	46·17	43·66	46·80	44·84
Crude fibre	14·24	16·44	15·51	17·28	20·82	18·93
SiO_2-free ash	6·20	7·16	6·64	6·17	7·35	6·82
True protein	18·14	22·75	20·35	17·86	21·83	20·59
"Amides"	2·40	3·84	2·01	2·18	3·98	2·84
Lime	1·36	1·98	1·68	1·22	1·67	1·44
Phosphoric acid	0·84	1·00	0·90	1·01	1·21	1·12
Moisture as cut	60·90	75·20	70·00	70·90	79·20	76·50

* Excluding results for drought period of late August and September, 1929.

It will be noted from Table II that, in the 1929 investigation, the monthly composite grass samples for April and early May contained, on the dry matter basis, 25·93, 22·66 and 21·75 per cent. of crude protein respectively, while the corresponding samples in the 1930 investigation contained 23·52 and 22·09 per cent. respectively. These values are not

very different from those which characterised the April and early May grass samples in the previous years of experiment, namely, 21·20 and 26·37 per cent. (1925 weekly cuts); 27·93, 25·48 and 23·49 per cent. (1927 fortnightly cuts); 24·89 and 22·69 per cent. of crude protein (1928 3-weekly cuts). For further comparison, it may be pointed out that the April herbage from the weekly cut control sub-plot contained, in its dry matter, 26·59 and 24·82 per cent. of crude protein in the seasons of 1929 and 1930 respectively.

It is evident, therefore, that pasture grass during April and early May contains, on the basis of dry matter, well over 20 per cent. of crude protein whether the system of cutting is weekly, fortnightly, 3-weekly or monthly; that is to say, its richness in protein is, within the limits of the investigations so far carried out, independent of the frequency of the cuts. The practical significance of this conclusion will be discussed later.

That grass cut at weekly and at monthly intervals during April is not only similar in respect of crude protein content, but also in respect of general composition, both organic and inorganic, is brought out strikingly in Table IV, in which is recorded the crude composition of the April samples of weekly cut grass (control sub-plot) and monthly cut grass for the seasons of 1929 and 1930.

Table IV. *Composition of April grass under weekly and monthly systems of cutting in the* 1929 *and* 1930 *seasons (dry matter basis).*

	Season 1929		Season 1930	
	April monthly cut grass %	April weekly cut grass %	April monthly cut grass %	April weekly cut grass %
Crude protein	25·93	26·59	23·52	24·82
Ether extract	7·06	6·90	3·43	3·75
N-free extractives	45·28	44·33	45·90	45·02
Crude fibre	14·04	14·24	17·72	18·16
Ash	7·69	7·94	9·43	8·25
Lime	1·31	1·36	1·30	1·22
Phosphoric acid	0·92	0·92	1·04	1·04

Although the data in Table IV show that there were very characteristic differences between the April grass samples in the two different years of experiment (differences which were, as will be made clear later, to be correlated with the marked differences in the growth conditions in the two seasons) yet it is clear that the weekly cut grass of April 1929 was scarcely distinguishable in general composition from the monthly cut grass obtained in the same month, and that further, the weekly and

monthly pasture cuts for April 1930 also displayed remarkable similarity in general composition. It may be concluded, therefore, that when a pasture has been closely grazed in the previous autumn, there is little danger of the herbage running off in composition during the April of the new season, even though the pasture is only lightly grazed during this month. The significance of the foregoing findings will be dealt with more fully in the section dealing with the digestibility and nutritive value of the monthly pasture cuts.

It will now be of interest to compare the seasonal changes in the composition of the 1929 weekly mown herbage with those of the monthly samples obtained in the same season. For this purpose, it will be advisable to omit the abnormal results obtained during the severe drought of late August and September. These results will be dealt with separately.

The trend of change in the crude protein content of the weekly cut herbage in the 1929 season is shown by the following values which were obtained (dry matter basis): 26·59 per cent. (April); 23·70 per cent. (May); 23·66 per cent. (June); 20·89 per cent. (July); 21·45 per cent. (August), the mean value over the whole period being 23·26 per cent. The corresponding data for the fibre content of the samples were 14·24, 14·64, 15·97, 16·26 and 16·44 per cent. respectively, with a mean value of 15·51 per cent. In the case of the monthly composite samples, it will be noted (see Table II) that the high crude protein percentage for the early April grass (25·93 per cent.) gradually fell off as the season advanced, the value remaining fairly constant between 18 and 19 per cent. during the second half of May, June and early July and thereafter rising to rather more than 19 per cent., the mean value over the whole period being 20·23 per cent. of the dry matter. The content of crude fibre displayed the usual inverse variation, being lowest in the April samples (13·20 to 14·04 per cent.) and highest during June (18·96 per cent.), the mean value being 16·92 per cent. of the dry matter. On the whole, therefore, the weekly cut herbage was some 3 per cent. richer than the monthly cut grass in respect of crude protein and, on the other hand, somewhat poorer in crude fibre. This constituted the main difference between the two kinds of herbage, and it is important to emphasise that, in the early part of the season, the distinction in this respect was but slight and only became marked with the advent of the "flush" period of growth, at which stage the monthly cut grass contained 18·71 per cent. of crude protein and 18·84 per cent. of crude fibre (dry matter basis), while the weekly mown herbage contained 23·66 and 15·97 per cent. respectively of these constituents. Reference to Table III reveals the

fact that the differences between the weekly and monthly samples in respect of ether extract, lime and phosphoric acid were not very appreciable, although, as would be expected, the monthly samples were, on the whole, slightly richer in N-free extractives than those obtained by cutting at weekly intervals.

The foregoing findings were confirmed in a qualitative sense by the results which were obtained in the 1930 season for the composition of the herbage samples from the unfertilised weekly and monthly control sub-plots (see Table III). A comparison of the 1930 results with those for the 1929 season, however, brings to light a finding of very great interest. The figures for the fertilised monthly pasture cuts of 1930 differ from those of the unfertilised monthly cuts of 1929 in two important respects, namely, the 1930 samples throughout were appreciably richer in crude fibre, and distinctly poorer in ether extract, than the samples obtained in 1929 (see Table II). Omitting the results for the late seasonal drought in 1929, it will be seen that the mean percentages of crude fibre and ether extract (dry matter basis) were 16·92 and 6·51 respectively in 1929, and 21·20 and 4·14 per cent. in 1930. Further, it is noteworthy that the percentages of crude protein in the 1929 samples were maintained at a slightly higher level than was the case with the 1930 samples.

To what factors are these differences to be attributed? It might be thought that they were connected with the intensive fertilising of the pasture plot during the 1930 season. That this is not the case, however, is evidenced by the fact that the herbage from the 1930 *unfertilised* monthly control sub-plot also differed from the 1929 pasture cuts in respect of fibre, ether extract and protein content in exactly the same manner as did the 1930 *fertilised* monthly pasture samples. In addition, it was found that the same characteristic differences in content of fibre and ether extract existed between the 1929 and 1930 *weekly* pasture cuts, both of which sets of samples were obtained without the use of fertilisers.

It is most probable that the differences are to be attributed solely to differences in the character of the two seasons, and if this be true, the danger of drawing conclusions on the basis of a single year's work is emphasised. The 1929 season was a period of slow and retarded growth, consequent on the droughty conditions which prevailed, whereas 1930, with its unusually favourable meteorological conditions, was a season of exceptionally quick and active growth (see Table XI). It is to be inferred, therefore, that in a quick-growing season such as 1930, characterised by greatly enhanced vegetative activity in pasture plants, the amount of ether extract in the herbage of pastures may be depressed

to a marked extent, and the crude protein to a slight extent, whereas the amount of crude fibre may be raised quite considerably. Whether this stimulation of fibre production is accompanied by a corresponding running off of digestibility and feeding value in the herbage will be revealed in the section dealing with the nutritive value of the 1930 monthly pasture cuts.

In seeking to trace any possible effects on the composition of the herbage arising from the intensive use of fertilisers, it should be pointed out that any conclusions in this respect must be regarded as purely tentative, since the results of one year's work only are available. The present investigation was not designed primarily to test the effects of nitrogenous fertilisers on the yield and composition of pasturage, this aspect of the pasture problem having been made the theme of a separate large-scale investigation, the results of which it is hoped to publish shortly. With these reservations, it may be pointed out that the use of fertilisers, including chalk, potash, phosphoric acid and nitrogen, does not appear to have produced, in the first year at any rate, any very pronounced change in the composition of the herbage. The fertilised herbage was, on the whole, slightly richer in crude protein, but the difference was not very significant (see Table III). In other respects, the organic composition of the fertilised and unfertilised monthly mown herbage was very similar. In regard to mineral composition, it will be noted that there was little to distinguish the two types of herbage so far as content of phosphoric acid was concerned. The herbage grown with the help of fertilisers was slightly poorer in lime than the samples secured from the unfertilised sub-plot, the mean percentages on the dry matter basis being 1·31 per cent. for the former and 1·41 per cent. for the latter. This difference, however, did not reveal itself in the first half of the season and only became evident from July onwards. It may, therefore, have been connected with the slightly poorer development of wild white clover on the main fertilised plot as compared with the unfertilised control sub-plot (see botanical notes). The difference in any case was so slight, however, that it is fair to conclude that the use of fertilisers on an intensive scale, on this particular pasture and in this particular year, was not attended, during the first season of their application, by any important changes in the chemical composition of the herbage.

SEASONAL VARIATIONS IN THE DIGESTIBILITY AND NUTRITIVE VALUE
OF THE MONTHLY PASTURE CUTS (SEASONS 1929 AND 1930).

The seasonal variations in the digestibility of the herbage obtained from the pasture plot under the system of monthly cuts were followed by carrying out a succession of digestion trials on pure-bred Suffolk wether sheep, aged about 13 months at the commencement of the season. During the 1930 season six separate digestion measurements were made, whereas in 1929 five digestion trials only were possible, the September trial being omitted owing to scarcity of herbage occasioned by the droughty nature of the season. The daily ration of herbage was 4000 gm. and during the 1930 trials there was no difficulty in maintaining the ration at this amount throughout the entire investigation. In the droughty periods 4 and 5 of the 1929 season, it was not possible on every day to feed the full ration to the sheep. The dryness of the herbage during these periods, however, brought up the dry matter content of the daily food supply to a satisfactory amount.

Table V. *Summary of digestion coefficients.*

(1) 1929 season (mean for sheep XI and XII).

Period... ...	1	2	3	4	5
Date (1929) ...	Apr. 20– Apr. 29	May 14– May 27	June 8– June 18	July 13– July 26	Aug. 11– Aug. 24
	%	%	%	%	%
Organic matter	82·6	79·3	76·6	71·4	72·3
Crude protein	79·4	74·7	73·8	73·9	74·2
Ether extract	62·3	71·1	58·1	35·5	45·3
N-free extractives	87·6	83·8	81·1	75·6	76·0
Crude fibre	77·6	75·9	74·7	69·5	70·6

(2) 1930 season (mean for sheep XIII and XIV).

Period... ...	1	2	3	4	5	6
Date (1930) ...	Apr. 15– Apr. 28	May 10– May 23	June 7– June 20	July 5– July 18	Aug. 9– Aug. 22	Sept. 6– Sept. 19
	%	%	%	%	%	%
Organic matter	83·4	80·7	77·6	76·7	80·3	79·2
Crude protein	82·1	76·0	75·3	75·9	77·7	79·5
Ether extract	42·4	55·9	48·8	33·2	44·5	37·0
N-free extractives	87·1	82·7	78·6	79·7	83·5	82·5
Crude fibre	83·7	84·9	82·2	78·6	82·9	81·2

The details of the digestion trials are recorded fully in the Appendix, and from these data it is possible, if required, to calculate the percentage composition of the composite samples of faeces and herbage appertaining to the separate digestion trials. The mean composition of the *soil-free* pasture cuts corresponding with the several digestion periods has already been given in Table II. The mean values of the digestion

coefficients of the herbage for the seasons 1929 and 1930 are summarised in Table V, while the data in respect of the digestible composition and nutritive value of the pasture cuts are recorded in Table VI.

Table VI. *Amounts of digestible nutrients in dry matter of monthly pasture cuts. Summary of starch equivalents and nutritive ratios.*

(1) 1929 season.

Period	1	2	3	4	5
Date (1929)	Apr. 20–	May 14–	June 8–	July 13–	Aug. 11–
	Apr. 29	May 27	June 18	July 26	Aug. 24
	%	%	%	%	%
Digestible crude protein	17·99	14·10	13·45	14·12	14·13
Digestible ether extract	3·53	5·47	3·95	2·10	3·07
Digestible N-free extractives	44·15	38·77	38·27	35·90	35·99
Digestible crude fibre	10·24	12·79	14·16	13·03	13·07
Digestible organic matter	75·91	71·13	69·83	65·15	66·26
S.E. per 100 lb. dry matter	74·11	70·37	67·11	60·77	62·83
Nutritive ratio	3·48	4·55	4·57	3·81	3·97

(2) 1930 season.

Period	1	2	3	4	5	6
Date (1930)	Apr. 15–	May 10–	June 7–	July 5–	Aug. 9–	Sept. 6–
	Apr. 28	May 23	June 20	July 18	Aug. 22	Sept. 19
	%	%	%	%	%	%
Digestible crude protein	19·31	13·07	12·98	13·18	14·20	15·49
Digestible ether extract	1·45	2·34	2·00	1·30	1·93	1·78
Digestible N-free extractives	39·98	38·15	35·68	39·03	39·25	38·21
Digestible crude fibre	14·83	19·82	19·97	16·47	17·96	16·45
Digestible organic matter	75·57	73·38	70·63	69·98	73·34	71·93
S.E. per 100 lb. dry matter	70·60	68·00	64·63	64·30	68·00	66·75
Nutritive ratio	3·01	4·85	4·64	4·44	4·34	3·79

Comments on Tables V and VI.

The significance of the data in Tables V and VI will be clearer if a comparison of these figures is made with the corresponding results obtained on the same pasture in earlier years under systems of weekly, fortnightly and 3-weekly cutting. A comparison of the digestion coefficients of the organic matter in the pasture samples under the different systems of cutting is set out in Table VII.

A consideration of the data in Table VII brings to light a striking fact, namely, that the high digestibility of pasture herbage in early spring is, within the limits of the experiments so far carried out, independent of the system of cutting. The digestion coefficients of the organic matter of the grass lay within the region of 82·1 to 83·4 per cent., whether the grass was cut at weekly, fortnightly, 3-weekly or monthly intervals. That the high protein content of early spring grass is independent of the frequency with which the plot is cut has already been demonstrated, and

the figures given in Table VIII show that the same holds true in respect of nutritive value.

Table VII. *Digestion coefficients of organic matter of pasture cuts. Comparison of results obtained in 1925, 1927, 1928, 1929 and 1930 on light-land pasture.*

Month ...	Apr. %	May %	June %	July %	Aug. %	Sept. %
1925 weekly cuts*	83·4	83·6	80·7–77·4	74·0	74·4–75·8	79·7
1927 fortnightly cuts*	82·3	81·4	78·6	78·0	78·3	79·3
1928 3-weekly cuts*	82·1	81·3	79·5	78·6	78·1	77·6
1929 monthly cuts*	82·6	79·3	76·6	71·4	72·3	—
1930 monthly cuts†	83·4	80·7	77·6	76·7	80·3	79·2

* No fertilisers applied to pasture.
† Under conditions of intensive fertilising.

Table VIII. *Nutritive value of April grass under different systems of cutting (dry matter basis).*

	Digestible protein %	Total digestible* carbohydrate %	Total digestible organic matter %	Starch equivalent %
1925 weekly cuts†	16·81	55·81	75·59	73·70
1927 fortnightly cuts†	20·82	48·74	74·54	73·75
1928 3-weekly cuts†	18·27	52·44	74·65	72·72
1929 monthly cuts†	17·99	54·39	75·91	74·11
1930 monthly cuts‡	19·31	54·81	75·57	70·60

* Digestible N-free extractives plus digestible fibre.
† No fertilisers applied to pasture.
‡ Under conditions of intensive fertilising.

It is clear, therefore, from the data tabulated in Tables VII and VIII, that the April samples of grass, obtained under systems of weekly, fortnightly, 3-weekly and monthly cutting are very similar in respect of digestible composition and nutritive value. The conclusion may be drawn that under a system of rotational grazing, the interval between successive grazings may be lengthened to a month and still the herbage during the early part of the season (April) will be equal in respect of digestibility, digestible protein content and starch equivalent to grass which is continuously and heavily grazed during this part of the year. This finding has an important application in grazing practice, in that it demonstrates that over-grazing of a pasture in the early part of the season is not necessary in order to ensure the best results from the standpoints of digestible protein content and nutritive value, *provided, of course, that the herbage was grazed down efficiently at the close of the previous season.*

This statement holds true for both fertilised and unfertilised pasture. Herein lies a dual advantage, since (1) heavy grazing in the earliest part of the season may react adversely on the persistence of certain early grasses and on the productivity of the pasture in the later stages of the season, and (2) a more lenient system of grazing in early spring leads to a more abundant yield of herbage at that particular time of the year, especially if the weather conditions are not entirely favourable to the growth of pasture grass.

It will be noted from Table VII that the digestion coefficients of the organic matter of the May monthly pasture cuts in both 1929 and 1930 were only slightly lower than the corresponding May values in 1925 (weekly cuts), 1927 (fortnightly cuts) and 1928 (3-weekly cuts). The falling-off in the values, particularly in 1930, was so slight as to be without important significance; indeed, the somewhat lower value for May 1929, namely, 79·3 per cent., may be merely a reflection of the unfavourable conditions for growth which prevailed in the early part of this season. The combined results of 1929 and 1930 suggest that during the month of May, as in April, there is little, in respect of digestibility of the total organic matter, to distinguish grass grown under systems of weekly, fortnightly, 3-weekly and monthly cutting. This is a significant conclusion, since during 1930 the second half of the May digestion period fell within the "zenith" growth period (May 17 to 31).

Considering the digestibility of the individual constituents of the grass, however, it would appear that during May there is a small, though definite, running-off in the digestibility of the crude protein in the monthly pasture cuts (see Table IX). The results for 1929 show that from the end of April onwards, the digestibility of the protein constituent in the monthly mown herbage was depressed to a lower level than was experienced in previous years when the herbage was cut at shorter intervals, the digestion coefficient falling as low as 73·8 per cent. in June (at a time when the grass was thin and stemmy) and never recovering beyond the value 74·2 per cent. recorded in August. That this depression, however, was partly a consequence of the unfavourable droughty conditions of the 1929 season is indicated by the results for 1930. During this more favourable season, the tendency of the crude protein in the monthly mown herbage to run off in digestibility was also noted, but not to such a marked extent, the digestion coefficient never falling below 75·3 per cent. and recovering ultimately in September to nearly 80 per cent.

The depression of protein digestibility noted in May and the succeeding months of 1929 was accompanied by corresponding changes in

the digestibility of the fibrous constituent of the herbage, the fibre digestion coefficients falling to 69·5 and 70·6 per cent. in July and August, with a contemporaneous falling-off in the digestion coefficients of the N-free extractives to 75·6 and 76·0 per cent. respectively. These changes are also reflected in the digestion coefficients of the total organic matter of the monthly pasture cuts for 1929, the values in this respect

Table IX. *Digestion coefficients of individual constituents in pasture cuts.* *Comparison of results obtained in 1925, 1927, 1928, 1929 and 1930 on* *light-land pasture.*

Month ...	Apr. %	May %	June %	July %	Aug. %	Sept. %
(1) Crude protein:						
1925 weekly cuts	79·3	85·4	81·1–78·4	77·1	76·6–78·9	83·4
1927 fortnightly cuts	81·7	79·3	78·8	79·2	79·5	80·7
1928 3-weekly cuts	80·5	78·1	78·4	79·4	80·2	78·8
1929 monthly cuts	79·4	74·7	73·8	73·9	74·2	—
1930 monthly cuts*	82·1	76·0	75·3	75·9	77·7	79·5
(2) Ether extract:						
1925 weekly cuts	60·5	60·0	53·5–51·2	39·7	47·9–55·1	54·9
1927 fortnightly cuts	66·0	59·2	52·7	54·6	53·7	51·9
1928 3-weekly cuts	60·5	61·4	58·3	48·4	50·5	48·6
1929 monthly cuts	62·3	71·1	58·1	35·5	45·3	—
1930 monthly cuts*	42·4	55·9	48·8	33·2	44·5	37·0
(3) N-free extractives:						
1925 weekly cuts	88·1	87·4	81·6–78·5	75·0	76·0–77·4	79·9
1927 fortnightly cuts	85·6	85·3	80·8	80·4	80·0	82·0
1928 3-weekly cuts	86·2	85·8	82·7	82·2	81·0	80·8
1929 monthly cuts	87·6	83·8	81·1	75·6	76·0	—
1930 monthly cuts*	87·1	82·7	78·6	79·7	83·5	82·5
(4) Crude fibre:						
1925 weekly cuts	80·3	79·2	84·2–80·0	76·1	76·0–75·1	80·4
1927 fortnightly cuts	82·1	81·1	81·3	80·3	82·3	81·1
1928 3-weekly cuts	81·1	79·4	79·5	78·5	77·5	77·4
1929 monthly cuts	77·6	75·9	74·7	69·5	70·6	—
1930 monthly cuts*	83·7	84·9	82·2	78·6	82·9	81·2

* Under conditions of intensive fertilising. No fertilisers applied in other years.

falling to 71·4 and 72·3 per cent. in July and August. It might be inferred from the foregoing results that the extension of the interval between successive cuttings to a month had permitted the herbage to reach the stage at which lignification sets in, with consequent decline of digestibility. This was probably the case in 1929, when the extreme dryness of the season reduced very considerably the rate of uptake of food material from the soil and the speed of the growth processes within the herbage plants, thus affording favourable conditions for the early onset of the lignification changes. In other words, the droughty conditions led to a premature cessation of the processes associated with the

vegetative phase of plant development, thus causing an early setting-in of the reproductive phase of activity, involving the re-transportation and rearrangement of materials already in the herbage plants, with consequent lignification of the cell walls. It will be noted, however, that during the moist, quick-growing season of 1930 quite different results were obtained. In this year the digestion coefficients of both crude fibre and N-free extractives remained at a high level throughout the whole season, comparing favourably with the values obtained in 1925, 1927 and 1928, when the herbage was cut at intervals of one, two and three weeks respectively. Further, the digestion coefficients of the total organic matter in the 1930 monthly pasture cuts did not fall below 76·7 per cent. in mid-season and recovered to 80·3 per cent. in August. The data for 1930 certainly do not suggest that the adoption of a monthly system of cutting had made possible the partial lignification of the herbage.

In the light of the results so far obtained in this series of investigations, the following general conclusion may be drawn: Pasture grass grown under a system of 3-weekly cuts is equal to weekly and fortnightly mown herbage in respect of digestibility. If the interval between successive cuts be lengthened to a month, the grass obtained in the early part of the season is as digestible as that obtained under systems of weekly, fortnightly and 3-weekly cutting. The effect of the longer interval on the digestibility of the herbage as the season advances will be determined largely by the weather conditions. If conditions are eminently favourable to quick growth, as in 1930, the digestibility of the herbage will remain high, as under more severe systems of cutting, the only noticeable effect being a slight running-off in the digestibility of the protein constituent during the mid-season, followed by recovery at a later stage. If, on the other hand, the season is substantially one of drought, with consequent slow growth, the herbage will tend, as the season advances, to suffer some degree of lignification and its digestibility will be lowered. In a season displaying more normal behaviour in respect of amount and distribution of rainfall, it is probable that the mid-seasonal falling-off in digestibility (followed by recovery in August and September) would not be so considerable, although perhaps, as is suggested by the results in Table VII, more pronounced than would be experienced under a system of cutting every three weeks.

It has been pointed out in an earlier publication[4] that dry mid-seasonal conditions give rise to a more pronounced depression of the digestibility of pasture herbage under a severe system of cutting than under a lenient system. There would appear to be, however, an optimum

leniency of cutting, probably, so far as can be ascertained at present, when the interval between successive cuts is three weeks. If the interval be lengthened beyond this optimum period, the consequence may be an intensification of the depressing influence of the dry mid-season on the digestibility of the herbage such as is brought about by shortening the interval. It is hoped to secure further information on this point in the coming season (1931).

It should be emphasised that the findings discussed above are applicable only to such pastures as are managed in a manner comparable with the systems of cutting adopted in these investigations, that is to say, pastures which are *efficiently and uniformly grazed down* at regular intervals throughout the season, the herbage being permitted to grow unchecked between the successive grazings. In order to secure in practice the nutritional advantages indicated by the early-season results in these investigations, it is imperative that pastures should be uniformly grazed down at the end of the pasture season. Finally, it is desirable to point out that, when necessary, the mowing machine is a useful adjunct to the grazing animal in enabling the desired conditions to be realised. For example, the farmer who decides to lengthen the interval between successive grazings to a month may find, in a quick-growing season such as 1930, that the herbage at certain periods tends to "grow beyond" the animals. During the "flush" period of growth, therefore, it may be necessary to shorten the interval between successive grazings or, alternatively, in order to prevent the herbage from advancing too far in maturity and thus prejudicing the nutritive value of the pasture in the subsequent period of the season, to take the mowing machine over the area before conditions become critical. Such mown herbage, if not consumed by pasturing animals at the time, should be conserved for feeding at a later stage of the season, when grass is not so abundant, or it may be reserved for use in winter (5).

The 1930 results throw an interesting light on the obscure question of lignification in herbage plants. Already in May, the second half of which marked the period of "zenith" growth, the fibre had risen as high as 23·35 per cent. of the dry matter. The value rose still further to 24·29 per cent. in June and thereafter never fell below 20 per cent. It will further be recalled that during the "flush" period of growth, perennial rye grass had attained a height of 8 to 9 in., with numerous stems, in the early flowering stage, up to 12 in. It might therefore have been anticipated that during this season the digestion trials would have revealed definite signs of lignification in the herbage. Such, however,

was by no means the case, as has been made clear in the foregoing account. The fibrous constituent was very highly digestible throughout the entire season and was manifestly composed almost wholly of the simple and digestible form of cellulose, unmixed with any significant amount of the indigestible lignocellulose.

It would appear that, up to the flowering stage, the magnitude of the fibre percentage in grass affords no certain indication as to whether lignification has, or has not, begun. It would manifestly be unsafe to base conclusions in respect of digestibility on the evidence of chemical composition alone. If the 1930 herbage had been permitted to grow unchecked for hay, instead of being cut at monthly intervals, the fibre content would have increased to about 32 per cent. of the dry matter (see Table XVIII). At this stage of maturity, the fibre would have displayed the usual degree of lignification associated with meadow hay. It is clear, if the argument be based on the findings of the 1930 investigation, that fibre production may go on in growing herbage until the amount is as high as 24 per cent. of the dry matter, without the chemical character of the fibre undergoing alteration. Throughout this period, the digestible form of cellulose only is being elaborated in the herbage plant. At some stage in the subsequent growth of the herbage, marked by the production of but a further 7 or 8 per cent. of fibre (dry matter basis), the character of the fibre being deposited changes from cellulose to lignocellulose, and the herbage becomes lignified. Lignification in herbage plants, therefore, is apparently delayed until the final stages of fibre production. The process does not occur during the vegetative phase of development, but begins only in the late-flowering stage, or even during the period of seed formation, when the stems and leaves are being depleted of nutrients.

If the lignocellulose which is deposited in the cell walls during this process is actually elaborated from simple nutrients, as is customarily assumed to be the case, the results suggest that the formation of quite a moderate amount is sufficient to cause lignification of the herbage. On the other hand, however, it is possible that the cellulose already present in the cell walls may itself partially undergo modification with the production of lignocellulose. Further experiments will be necessary before this point can be elucidated fully. Meantime, it should be made clear that the foregoing observations are based on results obtained in a season of particularly good conditions for active and continuous growth, and that the 1929 results indicate that if persistent drought leads to an untimely check or cessation in the growth of herbage, then the lignifica-

tion processes may set in at an earlier stage of distinctly lower fibre content than is indicated by the results of the quick-growing 1930 season.

Table X. *Showing comparison between digestible composition and nutritive value of herbage from light-land pasture under weekly, fortnightly, 3-weekly and monthly systems of cutting (dry matter basis).*

Season	1925 (4) Weekly			1927 (4) Fortnightly			1928 (4) 3-weekly		
System of cutting									
	Extreme values		Mean value	Extreme values		Mean value	Extreme values		Mean value
	%	%	%	%	%	%	%	%	%
Digestible crude protein	16·25	23·45	19·97	17·09	20·82	18·75	15·12	18·31	16·66
Digestible ether extract	1·96	3·55	2·87	3·16	4·98	3·81	2·93	3·94	3·38
Digestible N-free extractives	33·30	45·91	36·10	33·50	40·38	36·50	35·14	41·53	38·65
Digestible fibre	9·48	14·14	12·08	11·54	14·10	13·10	12·38	14·96	13·64
Digestible organic matter	66·89	75·59	71·02	70·20	74·54	72·16	70·50	74·65	72·33
S.E. per 100 lb. dry matter	62·08	73·70	67·74	67·79	73·75	69·87	67·15	72·72	69·39
Nutritive ratio	2·18	3·72	2·79	2·89	3·59	3·13	3·08	4·17	3·63

Season	1929† Monthly			1930* Monthly		
System of cutting						
	Extreme values		Mean value	Extreme values		Mean value
	%	%	%	%	%	%
Digestible crude protein	13·45	17·99	14·76	12·98	19·31	14·70
Digestible ether extract	2·10	5·47	3·62	1·30	2·34	1·80
Digestible N-free extractives	35·90	44·15	38·62	35·68	39·98	38·38
Digestible fibre	10·24	14·16	12·66	14·83	19·97	17·59
Digestible organic matter	65·15	75·91	69·66	69·98	75·57	72·47
S.E. per 100 lb. dry matter	60·77	74·11	67·04	64·30	70·60	67·05
Nutritive ratio	3·48	4·57	4·10	3·01	4·85	4·18

* Under conditions of intensive fertilising. No fertilisers applied in other years.
† Omitting September drought period.

Comments on Table X.

It has been concluded from the results of previous investigations (4) that grass obtained under a 3-weekly cutting system, while slightly less rich in digestible protein, is nevertheless equal in respect of total digestible organic matter and starch equivalent to grass grown under weekly and fortnightly systems of cutting, such differences in these respects as are discernible between the data for 1925, 1927 and 1928 (see Table X) being attributable to differences of seasonal variation, consequent on the different meteorological conditions experienced in the three seasons, rather than to a direct effect connected with the different systems of cutting. It will now be of interest to ascertain the effect of widening the interval between successive cuts to a month on the digestible composition and starch equivalent content of the herbage.

It has already been shown (see Table VIII) that during the earliest part of the season there are no significant differences, in respect of digestible composition and starch equivalent, between grass submitted

to cutting at weekly, fortnightly, 3-weekly and monthly intervals. Differences become apparent, however, as the season advances. In both 1929 (droughty season) and 1930 (plentiful rainfall), the percentage of digestible protein in the herbage fell to lower levels under the system of monthly cuts than was the case in the previous years when the plot was cut at shorter intervals. The lowest percentage of digestible protein in both seasons was encountered in mid-June, shortly after the period of "zenith" growth on the pasture, being 13·45 per cent. in 1929 (June 8 to 18) and 12·98 per cent. in 1930 (June 7 to 20). Even as early as in May, however, the content of digestible protein in both years had fallen to a value lower than the minimum values for the previous seasons in which the influence of more frequent systems of cutting had been investigated (see Tables VI and X). Despite the marked differences in weather conditions between the 1929 and 1930 seasons, the averages of the percentages of digestible protein determined over the whole period of experiment were remarkably similar in each year, namely, 14·76 and 14·70 per cent. of the dry matter during 1929 and 1930 respectively, the corresponding values for 1925 (weekly cutting), 1927 (fortnightly cutting) and 1928 (3-weekly cutting) being 19·97, 18·75 and 16·66 per cent. respectively. It is obvious, therefore, that as the system of cutting becomes more lenient, the percentage of digestible protein in the herbage suffers a gradual and continuous decline.

Judged on the basis of the 1930 results, it would appear that the adoption of the monthly interval between successive cuts had led to a very pronounced falling-off in the amount of digestible ether extract in the herbage, the mean percentage during the 1930 season being only 1·80 per cent. (dry matter basis) as compared with 3·38 per cent. and 3·81 per cent. in 1928 (3-weekly cutting) and 1927 (fortnightly cutting) respectively. That this, however, was due to an effect of the moist quick-growing season in 1930 rather than to any influence associated with the system of cutting, is apparent from the corresponding results in the dry 1929 season, when the mean percentage of digestible ether extract in the monthly mown herbage, namely, 3·62 per cent., compared satisfactorily with the values for 1927 and 1928. To a similar effect of season is to be attributed the high content of digestible fibre in the 1930 herbage, varying from 14·83 to 19·97 per cent. of the dry matter, with a mean value of 17·59 per cent., since it will be noted again that the 1929 monthly mown herbage differed very notably in this respect, the slow growth in this season being responsible for even lower percentages of digestible fibre than those which characterised the 3-weekly cuts of 1928.

No noteworthy differences are discernible respecting the amount of digestible N-free extractives in grass cut at 3-weekly intervals (1928) and at monthly intervals (1929 and 1930), the mean percentages for 1928, 1929 and 1930 being 38·65, 38·62 and 38·38 per cent. respectively of the dry matter. It is also clear from the data in Table X that the monthly cuts of 1930 were as rich in total digestible organic matter as the grass obtained in previous years under less lenient systems of cutting. The percentage of digestible organic matter in the 1930 monthly cut grass ranged from 69·98 to 75·57 per cent. of the dry matter, with a mean value of 72·47 per cent. The range of variation in this respect was strikingly similar during 1928 (3-weekly cutting), 1927 (fortnightly cutting) and 1925 (weekly cutting), the mean percentages for these years being 72·33, 72·16 and 71·02 per cent. respectively. The slightly lower mean value for the 1929 monthly mown herbage, namely, 69·66 per cent., was the result of the greater mid-seasonal depression of digestibility consequent on the unusually droughty conditions which prevailed in this season (see Table VII).

Despite the equality, in respect of total digestible organic matter, between the 1930 monthly cuts and the herbage obtained by more frequent cutting in the previous seasons, it is noteworthy that the starch equivalents of the dry matter in the 1930 herbage ran on a slightly lower level, namely, from 2 to 3 per cent. lower, than the corresponding values in the earlier years. The average of the starch equivalents for 1930 was 67·05, as compared with the corresponding value of 69·39 for the 3-weekly cuts of 1928. Obviously, therefore, the nutritive value of the digestible organic matter in the 1930 monthly cuts was somewhat lower than that of the digestible organic matter in the 1928 3-weekly cuts. The reason for this is clear. The 1930 herbage was distinctly poorer in digestible ether extract than the 1928 herbage. In calculating the starch equivalent of the herbage, 1 lb. of digestible ether extract is given a value equal to 1·91 times that of 1 lb. of digestible carbohydrate. It follows, therefore, that the difference in content of digestible ether extract is magnified when the starch equivalents are calculated. Further, the 1930 herbage was notably richer in crude fibre than the 1928 herbage (see Table III), in consequence of which the correction factor to be applied in the calculation of the starch equivalent of the 1930 herbage is correspondingly larger than in the case of the 1928 herbage. This circumstance leads to a further widening of the difference between the starch equivalents of the two types of herbage. It should be recalled, however, that the high fibre and low ether extract percentages in the

1930 herbage were largely an effect of the unusually good conditions for quick growth in that season and were not to be ascribed to the extension to a month of the interval between successive cuts. It is, therefore, probable that in a season of ordinary rainfall, in respect both of amount and distribution, the falling-off in the starch equivalent of monthly mown herbage, as compared with herbage cut at more frequent intervals, would be slight and would be evidenced mainly in a somewhat greater mid-seasonal range of depression. It may be inferred that this conclusion would hold also for pastures submitted to rotational grazing with monthly intervals of unchecked growth, provided, of course, that at each grazing the herbage was efficiently and uniformly eaten down.

NUTRITIVE RATIO OF MONTHLY PASTURE CUTS.

In previous communications(4) it has been shown that grass cut at weekly or fortnightly intervals is too rich in digestible protein to provide, *when it constitutes the sole ingredient of the diet,* a correctly balanced diet for any class of farm animal. The desirability of employing carbohydrate concentrates, instead of oil cakes, for supplementary feeding on closely-grazed pastures has accordingly been emphasised.

Although, with the adoption of a system of 3-weekly cuts, it was noted that the nutritive ratio of the herbage tended to widen (see Table X), the values for the 3-weekly pasture cuts were still significantly narrower than that of milk during the early part of the season (1928) and also in July, August and September. For the herbage obtained during these periods of the season, therefore, the earlier conclusions still held good respecting the advantages of using carbohydrate-rich foods, instead of oil cakes, for the supplementary feeding of pasturing animals. During May and June, however, the nutritive ratio of the 3-weekly mown herbage widened to a value almost equal to that of milk. At this stage, the grass was adapted to the requirements of young stock and was approaching a suitable balance for dairy cows yielding from 3 to 5 gallons of milk per day. For fattening stock, however, a supplement of carbohydrate-rich food would still be necessary in order to furnish such animals with a diet balanced according to scientific standards.

How is the question affected when the interval between successive cuts (or close-grazings) is widened to a month? It has already been made clear that in the earliest part of the season (April), provided the pasture was left in a grazed-down condition at the end of the previous season, there is little difference in digestible composition and nutritive

value between grass cut (or grazed) at monthly intervals and grass submitted to cutting (or grazing) at shorter intervals. The dry matter in such monthly cut herbage during April contains nearly 20 per cent. of digestible protein and more than 70 per cent. of starch equivalent (see Table VIII) and is still to be regarded as a protein-concentrated food. Obviously, at this stage, supplementary food must take the form of carbohydrate-rich feeding stuffs.

During May and June, however, under a monthly system of cutting, the nutritive ratio of the herbage widens considerably. During the 1930 season, for instance, the nutritive ratios of the herbage were 4·85 and 4·64 respectively for May and June. Over this period, which is important because it includes the "flush" period of growth, the 1930 herbage contained, on the basis of dry matter, about 13 per cent. of digestible protein and about 66½ per cent. of starch equivalent. The balance of the "concentrate" dry matter at this stage, therefore, is much more suitable for general feeding purposes than was the case in previous years under more frequent systems of cutting. A few examples will serve to make this point clear.

According to the accepted feeding standards, a 5-gallon cow requires, in its daily ration, 19 lb. of starch equivalent, including 3·7 lb. of digestible protein. A ration containing 30 lb. of the dry matter of the 1930 May and June herbage would supply nearly 20 lb. of starch equivalent, including 3·9 lb. of digestible protein. Since during this period of abundant growth a dairy cow would have experienced no difficulty in securing a full ration from the pasture, it is clear that during May and June, monthly cut (or grazed) herbage is just rich enough in starch equivalent and digestible protein to furnish the requirements of a 5-gallon cow, and, indeed, to supply a little extra starch equivalent to make up for the energy expended by the animal in securing the herbage.

It will be noted that the foregoing statement is based on the assumption that the dairy cow is actually able to consume a daily ration of 30 lb. of dry matter in the form of grass. If it is able to consume more than this amount, then it will exceed the standards which have been accepted as requisite for the production of 5 gallons of milk. If, on the other hand, it is unable to eat as much as 30 lb. of dry matter in the form of pasture herbage, then a less bulky supplement must be fed. The necessity for feeding such a supplement would be apparent if the animal displayed signs of loss of condition while subsisting on the pasturage. In such a case, the supplementary food must obviously have the same content of digestible protein and starch equivalent as the grass which it

replaces, that is to say, it must contain about 13 per cent. of digestible protein and about 66 per cent. of starch equivalent. Supposing, for example, a 5-gallon cow consumes an amount of such herbage containing 25 lb. of dry matter, then its further requirements would be met by 4 lb. of a supplement composed of equal parts of Egyptian undecorticated cotton cake and flaked maize.

It has been shown that 30 lb. of dry matter in the form of May and June grass, cut or closely-grazed at monthly intervals, furnishes almost exactly the requirements of a 5-gallon cow. For animals yielding more than 5 gallons, it would appear logical to assume that the supplementary food on such pasturage should take the form of a concentrate mixture balanced for milk production, fed at the rate of $3\frac{1}{2}$ lb. for every gallon above 5 gallons. But feeding along these lines would involve difficulties arising from the size and bulk of the ration, since grass grazed at monthly intervals, though supplying dry matter with the character of a concentrate, is nevertheless, from the volume standpoint, a fairly bulky food. An 8-gallon cow, for example, could scarcely be expected to consume $10\frac{1}{2}$ lb. of a milk-production mixture in addition to 30 lb. of dry matter in the form of monthly grazed herbage. Hence arise the difficulties invariably met with when it is attempted to maintain the yield of very high-yielding cows on such pasturage. Little difficulty arises until the yield rises beyond 5 gallons. Beyond this limit, however, the concentrated food secured from rotationally-grazed pastures (monthly intervals) tends to become too bulky for the purpose, and the wise dairyman in such cases relies less and less on the grass, and more and more on the less bulky concentrated foods, as the yield of milk increases more and more beyond 5 gallons per day.

In the case of dairy cows yielding less than 5 gallons per day, pasturage closely-grazed at monthly intervals is able, as has been shown, to supply more starch equivalent and digestible protein than are required by the feeding standards. In the unlikely event of grass being scarce during the period of the season under consideration, namely, May and June, and the animal is unable to secure sufficient herbage for maintenance and milk production, the question arises as to the kind of supplement which should be provided. The case of the 3-gallon cow, of 10 cwt. live-weight, may be considered. Such an animal requires, per day, 14 lb. of starch equivalent, including 2·5 lb. of digestible protein. Supposing it is able to secure a half ration of grass, say 16 lb. of dry matter in the form of the monthly grazed herbage. This would supply, according to the 1930 data for May and June, about 10·6 lb. of starch

equivalent, including 2·1 lb. of digestible protein. The animal would therefore require a supplement containing 3·4 lb. of starch equivalent, including only 0·4 lb. of digestible protein, and this further requirement would approximately be met by 4 lb. of barley and a few lb. of indifferent meadow hay, the latter being included to increase somewhat the bulk of the ration. It is clear, then, that the correct supplements during May and June for cows yielding less than 5 gallons of milk on pastures closely-grazed at monthly intervals are the carbohydrate-rich, and not the protein-rich feeding stuffs.

The correct kind of supplement for fattening animals during the quick-growing part of the season on pastures submitted to a monthly rotational close-grazing system is again the carbohydrate-rich concentrate. Fattening cattle at all live-weights from 6 to 11 cwt. require no more than about 1½ lb. of digestible protein in their daily rations. This amount would be contained in about 11½ lb. of the dry matter of the herbage, and it follows, therefore, that the extra starch equivalent required by the fattening beasts need not be given in the form of oil cakes, but is best supplied in the form of carbohydrate foods such as maize, flaked maize, maize germ cubes or, better still, home-grown cereals and potatoes. It should be noted that the ordinary feeding standards, when applied to pasturing animals, are probably slightly on the low side, since manifestly an animal at pasture must expend rather more energy, in walking about in search of its food and in biting off the herbage, than it would use up when being fed under indoor conditions. Any extra small amount of starch equivalent required to furnish energy for muscular activity of this nature is most economically supplied in the form of carbohydrate concentrates.

An inspection of the data for 1930 in Table VI shows that the foregoing observations respecting the May and June herbage hold substantially for the herbage obtained in July and August. The main difference lies in the fact that herbage will not be so abundant at this later stage of the season, and greater attention must therefore be paid to the supplementary feeding of pasturing stock. During September, the herbage becomes distinctly richer again in respect of digestible protein, and the need for carbohydrate supplements is consequently more obvious at this stage. In the light of the figures obtained in the 1930 investigation, the advantages of having a heavy-yielding carbohydrate-rich crop like green maize, for supplementary feeding on such pasturage during late summer and early autumn, are manifest.

PRODUCTION OF NUTRIENT MATTER FROM PASTURE PLOT UNDER SYSTEM OF MONTHLY CUTTING (SEASONS 1929 AND 1930).

The following main conclusions in respect of the productivity of pastures under different systems of cutting or close-grazing (weekly to 3-weekly intervals) have been drawn from the results of the earlier investigations. If contiguous plots on a pasture be submitted to cutting at weekly, fortnightly and 3-weekly intervals, the plot cut every three weeks will be found to yield more heavily, in terms of lb. of dry matter per acre, than the fortnightly cut plot, while the productivity of the latter will be higher than that of the plot cut every week. Further, these disparities in productivity will be most marked at those times of the season when the conditions for growth are unfavourable, as, for example, during a spell of dry weather. Since there is little difference, from the standpoint of starch equivalent, between pasture herbage grown under systems of weekly, fortnightly and 3-weekly cutting, the foregoing finding is of great practical significance, in that it demonstrates an important advantage which "rotational close-grazing" has, from the standpoint of production of nutrient food, over "non-rotational or continuous close-grazing." It was shown, for example, that if the management of the experimental pasture had been attempted along the lines of close-grazing under the weather conditions of 1928, a simple division of the main field into a suitable number of smaller enclosures, for rotational close-grazing at 3-weekly intervals, would have enabled the stock-carrying capacity of such unfertilised pasture to be increased in the ratio of 2:3.

The results of previous investigations have emphasised the conclusion that the productivity of pastures is controlled primarily by meteorological factors, of which the most important is the rainfall, in particular, its distribution over the season. When, however, the weather conditions as a whole are unfavourable during any particular season, the yield will be influenced considerably by the degree of intensity of cutting or grazing.

In Table XI are given the total yields of herbage, in terms of lb. dry matter per acre, which were obtained from the pasture plot by cutting at monthly intervals during the seasons of 1929 and 1930.

It will be noted from Table XI that the difference between the yields from the pasture in the seasons of 1929 and 1930 was very striking, although in both years the system of cutting was the same. During 1930, the plot produced 2·67 times as much dry matter as was obtained during 1929. It has been shown, however, that growth on pastures

during the 1929 season was unusually restricted on account of the droughty conditions which prevailed. The predominant influence of the adverse weather conditions of 1929 is emphasised by the fact that during the more favourable season of 1925 the plot produced a much heavier yield of dry matter, namely, 2833 lb. per acre, under a system of *weekly* cutting than was obtained by *monthly* cutting during 1929.

Table XI. *Production of dry matter from light-land pasture plot by monthly cutting during* 1929 *and* 1930.

1929 (Apr. 12 to Oct. 3)* lb. dry matter per acre	1930 (Apr. 12 to Oct. 3)† lb. dry matter per acre
2010	5365

    * No fertilisers applied to pasture.
    † Under conditions of intensive fertilising.

The very marked improvement in productivity noted in 1930 was partly to be ascribed to the excellent conditions for growth which prevailed during that season and partly to the use of fertilisers. In order to partition the effect between these two factors, it will be necessary to consider the figures in Table XII, which records the yields of dry matter from contiguous sub-plots under different conditions of treatment.

Table XII. *Comparison of yields of herbage from contiguous sub-plots during seasons of* 1929 *and* 1930 (*yields given in terms of lb. dry matter per acre obtained from April* 12 *to beginning of October*).

Season 1929		Season 1930		
Sub-plot 10 (Unfertilised and cut every week) lb. dry matter per acre	Mean of sub-plots 11 and 12 (Unfertilised and cut every month) lb. dry matter per acre	Sub-plot 10 (Unfertilised and cut every week) lb. dry matter per acre	Sub-plot 11 (Unfertilised and cut every month) lb. dry matter per acre	Sub-plot 12* (Intensively fertilised and cut every month) lb. dry matter per acre
1454	2114	2782	4029	5762

    * For details of fertilising, which included dressings of chalk, sulphate of potash and superphosphate of lime, with periodic small applications of sulphate of ammonia, see an earlier section of this paper.

An insight into the magnitude of the influence of weather on the productivity of the pasture may be gained by comparing the yields in Table XII for the monthly cut, unfertilised sub-plot in 1929 and 1930. The contrasting meteorological conditions in these seasons were reflected in an increase of yield from 2114 lb. in 1929 to 4029 lb. dry matter per acre in 1930, an improvement equal to 90·6 per cent. of the lower yield. A very similar increase was noted in the yield of the weekly cut, un-

fertilised sub-plot 10, the improvement in 1930 being equal to 91·3 per cent. of the lower yield in 1929.

The combined influence of both season and fertilisers is revealed by a comparison of the yields of the monthly cut, unfertilised sub-plot in 1929 and the monthly cut, intensively fertilised sub-plot 12 in 1930. Here the improvement was equal to about 173 per cent. of the lower yield, that is to say, rather less than twice the improvement brought about by the influence of weather conditions alone. The direct stimulation of productivity by fertilisers alone is discernible in the yield differences during 1930 for the monthly cut, unfertilised sub-plot 11 and the monthly cut, intensively fertilised sub-plot 12, the latter producing 43 per cent. more dry matter than the former. It is of interest to compare this effect with that arising solely from altering the system of cutting from weekly to monthly cuts (sub-plots 10 and 11 in the 1930 season), the monthly cut, unfertilised sub-plot producing 44·8 per cent. more dry matter than the weekly cut, unfertilised sub-plot.

From the data in the foregoing paragraph, it may be inferred that the intensive fertilising brought about a percentage improvement of yield almost equal to that which was produced by cutting at monthly instead of weekly intervals. Further, the stimulation of yield by fertilisers in 1930 was not far short of that manifested on the unfertilised pasture sub-plots as a consequence of the change-over from unfavourable weather conditions in 1929 to favourable conditions in 1930. This striking effect of fertilisers on the yield, however, must be interpreted with caution. In the first place, the lay-out of the present experiments is manifestly not designed to bring out, with the desired degree of significance, the magnitude of the influence of fertilisers on the yield of pastures. More significant results are being obtained in a separate investigation, which has been planned with the primary object of testing this and kindred points. The results of this separate investigation will be published in due course. Further, in the trials of 1930, fertilisers were being employed for the first time on a pasture which, in previous years of work, had probably suffered considerable depletion in respect of its soil reserves. The conditions, therefore, were favourable to bringing out, in a striking manner, the stimulating effect of artificial fertilisers, more particularly as the abundant rainfall of 1930 enabled the periodic dressings of sulphate of ammonia to be brought readily into solution in the soil and thus to exert a quick effect on the growth of the herbage. Nothing further, for these reasons, will be written at this stage respecting the influence of intensive fertilising on pasture

productivity, the fuller treatment of this aspect of the question being left to a future publication dealing specifically with this question.

It has already been pointed out that the results of previous investigations have shown that the disparity in yield between plots cut with different frequencies is magnified when the weather conditions are unfavourable to growth on the pasture. It would be anticipated, therefore, that the yield difference between the unfertilised weekly cut and monthly cut sub-plots during the droughty season of 1929 would be very much more marked than the corresponding difference in the quick-growing 1930 season. Such was by no means the case, however. The differences, expressed in both cases as a percentage of the lower yield of the weekly cut sub-plot, were, in point of fact, almost equal, being 45·4 per cent. in 1929 and 44·8 per cent. in 1930.

It is necessary, therefore, to explain why the yield results for 1929 should constitute an apparent contradiction of the generalisation that the slowing-up of growth on pastures by unfavourable meteorological conditions leads to an exaggeration of the yield differences between plots submitted to cutting at short and at longer intervals. If the yield data for 1929 be examined in detail, the explanation becomes clear. It is found that after mid-July, growth had almost ceased on both the weekly cut and monthly cut sub-plots as a consequence of the prolonged drought and heat which prevailed during the second half of this season of investigation. Of the season's total yield of dry matter, namely, 1454 lb. per acre, from the weekly cut sub-plot, 1365 lb. had been produced by July 14. In the case of the monthly cut sub-plot, 1978 lb. of the season's total yield of 2114 lb. of dry matter per acre had already been obtained by this date. Further evidence of the abnormally slow growth during the second half of the 1929 season is afforded by the almost negligibly small yield of aftermath, namely, 140 lb. of dry matter per acre, which was obtained from the meadow sub-plot in this season (see Table XVI).

If, as is clear from the foregoing statements, the second half of the 1929 season made only an insignificant contribution to the season's total yield of dry matter from the sub-plots, it is clear that the figure 45·4 per cent., representing the disparity in total yield between the monthly cut and weekly cut sub-plots during this season, has reference in reality to the difference in yield up to July 14, after which date both sub-plots produced very little extra herbage. Up to July 14, the monthly cut sub-plot actually had produced 44·9 per cent. more dry matter than the weekly cut sub-plot. It follows that the severe drought of the second half of the 1929 season was almost entirely without effect on the magni-

tude of this difference figure; in effect, the season of 1929 ended on July 14. It is scarcely surprising, therefore, that the yield disparity between the monthly cut and weekly cut sub-plots for this *half-season*, in which the "flush" period was responsible for the main portion of the herbage produced, should be of the same order as, and not very much larger than, the difference between the corresponding sub-plots over the *whole season* of 1930.

The question now arises as to what light the present series of investigations throws on the problem of deciding under what system of cutting, or rotational grazing, does a pasture yield the maximum amount of nutrient food in a season. The question of this maximum yield is bound up with the investigation of the process of lignification in the herbage, the necessary conditions being realisable when the interval between successive cuttings, or close-grazings, is as long as possible. The length of this optimum interval will naturally depend on the time required by the young shoots of grass to reach the stage of growth at which lignification sets in. It may be taken for granted that, in order to maintain the highest possible yield season after season, attention must not only be directed to ascertaining the optimum interval between successive grazings, but also to discovering the most efficient system of fertilising the pasture.

Table XIII. *Seasonal variations in the yield of nutrient matter from the light-land pasture in 1930 under conditions of monthly cutting and intensive fertilising.*

Period of season (1929)	lb. dry matter per acre	Percentage of season's total yield of dry matter	lb. starch equivalent per acre	lb. dig. protein per acre
Apr. 12 to Apr. 30	453·3	8·4	320·0	87·5
May	1671·8	31·2	1136·8	218·5
June	1207·0	22·5	780·1	156·7
July	474·6	8·8	305·2	62·6
August	891·3	16·6	606·1	126·6
Sept. 1 to Oct. 3	667·0	12·5	445·2	103·3
Total	5365·0	100·0	3593·4	755·2

It will be of interest, therefore, to re-examine the results of the 1930 season in this light, since in this case not only was the system of cutting more lenient than was adopted in the earlier investigations, but the pasture was also intensively fertilised. It has already been demonstrated in a previous section of this paper that the lengthening to a month of the interval between successive cuts had very little effect on the starch equivalent and digestible protein content of the herbage during April

(see Table VIII). But the amount of herbage obtained at this stage represented merely 8·4 per cent. of the season's total yield, as is clear from the data in Table XIII, giving the seasonal yields of nutrient matter from the pasture plot under the conditions of the 1930 investigation.

The figures in Table XIII clearly indicate that it is of greater importance to take into account the digestible protein and starch equivalent content of the herbage during May and June, since over this period was produced approximately 54 per cent. of the season's total yield of dry matter. A simple calculation shows that the dry matter of the herbage at this stage of the season contained, on an average, 66·6 per cent. of starch equivalent, including 13 per cent. of digestible protein. The conclusion has already been drawn in an earlier section of this paper that during this quick-growing part of the season, the percentage of digestible protein in herbage cut at monthly intervals falls to a lower level than is reached when the pasture is submitted to more frequent cutting, although, at the same time, there is no significant falling-off in the percentage of total digestible organic matter or the starch equivalent content. The results in Table V for the digestion coefficients of the fibrous constituent of the 1930 monthly pasture cuts have further made clear that in a season of adequate and well-distributed rainfall no lignification should occur in herbage closely-grazed at monthly intervals. The balance of the "concentrate" food from the pasture under such a system of grazing is much more suitable for general feeding purposes than is the case when the pasture is submitted to a more severe system of cutting or grazing.

That the dry matter of the herbage from pastures closely-grazed under conditions comparable with those which obtained in the 1930 trial partakes of the character of a concentrated food is seen by a study of the data in Table XIII. On the basis of an acre, the pasture produced, during the season of experiment, 5365 lb. of dry matter, containing 3593 lb. of starch equivalent and 755 lb. of digestible protein. On an average, therefore, the dry matter contained 67 per cent. of starch equivalent, including 14·1 per cent. of digestible protein. In order to maintain the concentrated nutrient matter in the herbage at a higher level of digestible protein content, it would be necessary to cut, or close-graze, at shorter intervals. By adopting this procedure, however, the grazier would have to be prepared to make a sacrifice in respect of the yield of starch equivalent, which is a measure of the amount of "keep" for his animals. This is made clearer by the data recorded in Table XIV.

Table XIV. *Season's yields of nutrient matter in* 1930 (*April* 12 *to beginning of October*) *from contiguous sub-plots* 10, 11 *and* 12.

System of treatment	Sub-plot 10 Weekly cutting without application of fertilisers (lb. per acre)	Sub-plot 11 Monthly cutting without application of fertilisers (lb. per acre)	Sub-plot 12 Monthly cutting under conditions of intensive fertilising (lb. per acre)
Dry matter	2782	4029	5762
Starch equivalent	1947*	2699†	3860
Digestible protein	556*	568†	812

* Assuming weekly cut herbage to contain 70 per cent. of starch equivalent, including 20 per cent. of digestible protein.

† An approximate estimate on assumption that the monthly cut fertilised and un-fertilised grass have the same average nutritive value, *i.e.* 67 per cent. of starch equivalent, including 14·1 per cent. of digestible protein. The justification for this assumpion is based on the similarity of the data for the organic composition of the two types of herbage (see Table III).

It will be noted from Table XIV that the mere extension of the interval between successive cuts from a week to a month led to an extra production over the season of 752 lb. of starch equivalent per acre, *i.e.* a 38·6 per cent. improvement on the basis of the lower figure. Further, although the dry matter of the weekly cut herbage contains a distinctly higher percentage of digestible protein than that of the monthly cut herbage, yet the absolute amount of digestible protein produced in the herbage per acre during the season was almost the same under both systems of cutting. When, however, the monthly system of cutting was combined with conditions of intensive fertilising (sub-plot 12), the improvement of yield over that given by the weekly cut, unfertilised sub-plot 10 amounted not merely to 1913 lb. starch equivalent per acre (98 per cent. increase), *but also to an increase of* 256 *lb. of digestible protein per acre* (46 per cent. increase). This may be taken as a measure of the difference in productivity which would have been noted, *under the weather conditions of* 1930, if, instead of submitting the pasture to heavy, continuous close-grazing, without application of artificial fertilisers, it had been divided up into smaller enclosures for rotational close-grazing at monthly intervals, the close-grazing being supplemented by intensive fertilising.

Bearing in mind what has been written in an earlier section respecting the particularly favourable conditions obtaining on the experimental pasture for demonstrating the stimulating effect of artificial fertilisers on productivity, it is nevertheless justifiable to conclude that, within the limits of the systems of cutting so far investigated, monthly rotational close-grazing, reinforced by a suitable system of fertilising, provides

the optimum conditions for the maximum yield of starch equivalent (*i.e.* of "keep") from pastures. It should be emphasised, however, that the present investigation in no way attempts to decide what is the ideal method of fertilising pastures. Monthly-rotational grazing has the further advantage of conferring on the herbage a "balance" which renders it much more suited to form the sole diet of farm animals.

Since the 1929 results have revealed the tendency of monthly mown herbage, in a season of droughty, slow-growing conditions, to undergo slight lignification as the season advances, it must be inferred that the trend of the results appears to indicate that if the interval between successive close-grazings be extended to five weeks, the herbage will be enabled to reach the stage of growth at which lignification sets in, with consequent depression of digestibility and nutritive value. A definite pronouncement on this point, however, must await the results of a further investigation to be carried out in 1931, when it is intended to examine the yield, composition and nutritive value of pasturage under a system of 5-weekly cuts.

That the yield of 5365 lb. of dry matter per acre obtained from the light-land pasture under conditions of monthly cutting and intensive fertilising by no means represents the full productive capacity of pastures under such conditions of treatment is shown by the results of a separate trial on heavy-land pasture carried out in the same season (1930) and under precisely similar conditions of experiment. In this case, the yield of dry matter per acre from the beginning of April to mid-October was as high as 7996 lb. This is a very significant result in connection with the proposals for conserving the produce of pastures in the form of dried grass cakes(5). Allowing 8 per cent. of moisture in the conserved product, the figure represents a production of 3·85 tons of dried grass cake per season from an acre of such grassland. When cutting grass for conversion into such fodder-cake, however, it is important to remember that the yield and the "balance" of the resulting concentrate will vary with the frequency with which the grassland is cut, as has been made clear in this series of publications.

INFLUENCE OF PROLONGED DROUGHT ON THE COMPOSITION AND NUTRITIVE VALUE OF PASTURE HERBAGE.

It is usually thought that when pastures become "scorched" by prolonged heat and drought, as was the case in the late summer and early autumn of 1929, store animals may be able to make slow progress

on the scanty, brown herbage, but the yield of dairy cows soon begins to fall. In relation to this question, therefore, the following statement, based on observations during the severe drought of the 1929 season, is of particular interest[1]. "Over large areas of the country the drought remained unbroken until the end of September. For many weeks pastures have been brown and scorched and bare. Yet most farm animals throve remarkably well considering the circumstances. What can be the explanation? Some people say that animals have been less disturbed by fly pests than in other years, but flies have been less troublesome generally this year, and in parts of the North, where rain has been plentiful and grass abounds, it is doubtful whether sheep, for instance, are looking as well as those on some of the dried-up pastures of the South. Is it not possible that the quality of the grass may supply some part of the explanation? The work of Dr Woodman and others at Cambridge has shown that short grass, both in fresh and in artificially-dried condition, has a very high feeding value. Pastures in the South have been short all through the summer, and for lack of rain the herbage has been eaten in air-dried form. It is true that the stock-carrying capacity of the fields has been low, and that where the stocking has been heavy, animals have suffered from too little food. The conclusion surely must be that such grass as has been available has had a very high nutritive value."

Table XV. *Showing influence of severe drought on the composition of the monthly pasture cuts (dry matter basis).*

Composite sample ...	1	2	3
Date of cutting (1929) ...	Aug. 11–Aug. 24	Aug. 25–Sept. 20	Sept. 21–Oct. 3
	%	%	%
Crude protein	19·04	15·46	13·63
Ether extract	6·78	5·29	5·03
N-free extractives	47·36	51·34	52·42
Crude fibre	18·51	19·79	20·10
Ash	8·31	8·12	8·82
True protein	16·17	13·46	12·00
"Amides"	2·87	2·00	1·63
Lime	1·73	2·56	2·04
Phosphoric acid	0·99	0·82	0·76
Moisture as cut	62·90	55·00	49·70

The hypothesis put forward in the foregoing statement is naturally of the highest importance, and it is therefore of interest to ascertain what support is given to it by the results of the present investigation, since, as has already been explained, the experimental pasture, during

[1] See the *J. Min. Agric.,* "Notes for the Month" (Nov. 1929), **36**, 698.

the drought of August and September, 1929, presented a brown and scorched appearance. In Table XV are given the results of the analysis of the two composite grass samples obtained during the period when the grass was brown and scorched (samples 2 and 3), while, for comparison, the data for the immediately preceding composite sample, obtained before the full effects of the drought had been manifested, are also recorded in the table (sample 1).

The figures in Table XV disclose the main effects of the severe drought and the consequent "browning" of the herbage to have been as follows: (1) a very decided falling-off in the percentage of protein; (2) a slight increase in the percentages of N-free extractives and crude fibre; (3) an abrupt rise in the percentage of lime, accompanied by a decline in the percentage of phosphoric acid; (4) a pronounced reduction of the moisture content of the herbage at the time of cutting.

It may be pointed out that similar findings were obtained in respect of the influence of the drought on the composition of the herbage from the weekly cut sub-plot 10. In this case, the herbage of September contained, on the dry matter basis, 15·51 per cent. of crude protein, 52·63 per cent. of N-free extractives, 17·35 per cent. of crude fibre, 2·70 per cent. of lime and 0·75 per cent. of phosphoric acid. The mean moisture content of the grass at the time of cutting was as low as 36 per cent.

All these changes are such as might have been anticipated. It has been demonstrated in this series of investigations that the effect of the dry weather which is usually encountered during the mid-season is to lead to a temporary slight fall in the percentage of protein, a corresponding temporary increase in the percentages of crude fibre and N-free extractives, a rise in the percentage of lime to its maximum value for the season and a contemporaneous slight diminution in the percentage of phosphoric acid. The changes which were noted in the case of the grass samples for late August and for September in 1929 were an accentuation, particularly in respect of protein content, of these known effects, as a consequence of the continued prevalence of such hot, droughty conditions as ordinarily are encountered only over a short period during mid-season.

Owing to the very small yields of the brown, scorched herbage from the sub-plots in September 1929, it was unfortunately impossible to investigate its digestibility and nutritive value by direct animal experiment. Valuable information, however, was obtained in artificial determinations of the digestibility of the crude protein in the September

samples by means of the action of pepsin-HCl at 37° C. It was found that the protein digestion coefficient of sample 2 (August 25 to September 20) had fallen as low as 56 per cent., whilst that of sample 3 (September 21 to October 3) had declined to a still lower level, namely, 52 per cent.

These figures are indicative of a decided running-off in the digestibility and nutritive value of the monthly pasture cuts during the droughty period under investigation. It is not likely that the pronounced decrease in the digestibility of the protein in the grass is to be attributed to any alteration in the chemical character of this constituent, but is rather to be put down to the inevitable reduction of the digestibility of the food nutrients, contained in the plant cells, which accompanies lignification of the fibrous cell walls. It has been shown, in the section dealing with the nutritive value of the 1929 and 1930 herbage, that under a system of monthly cuts no evidence of lignification is manifested if the weather conditions are such as to encourage active and continuous growth, but that if lack of rainfall leads to a slowing-up in the rate of growth, then a stage may be reached, within the monthly interval between successive cuts, when the lignification processes will begin to modify the character of the herbage. This probably occurs, on an intensive scale, during a drought which is of such duration as to cause the herbage to become parched and brown. Transportation of fresh food material from the soil into the herbage plants becomes impossible owing to lack of the necessary moisture, and, as a consequence, the vegetative phase of plant development comes to an untimely end. The processes of re-transportation and re-elaboration of material already within the plant, characteristic of the reproductive phase of development, set in prematurely, one result of these operations being the gradual lignification of the cellulose in the cell walls. If this explanation be correct, it would follow that not only the protein, but also the fibre, N-free extractives and ether extract in the brown herbage would be of low digestibility, and the material would have a correspondingly low feeding value. Incidentally, such an explanation would appear to support the view that during lignification the cellulose in the cell walls may itself, in part, undergo modification with the formation of lignocellulose, since the percentages of crude fibre in samples 2 and 3 are only slightly higher than that in sample 1 (see Table XV).

Yield and composition of hay and aftermath
(Seasons 1929 and 1930).

In Tables XVI and XVII are recorded the data for the yields and composition of the hay and aftermath cuts from the meadow plot during the 1929 and 1930 seasons. It has already been explained that no fertilisers were employed on this plot during 1929, but that for the purposes of the 1930 work it was divided into three equal sub-plots, each comprising $\frac{1}{60}$ acre (meadow sub-plots 1, 2 and 3). In the November of 1929 a dressing of 5 tons per acre of well-rotted farm yard manure was applied to the whole of the meadow plot. Later, during December, meadow sub-plots 1 and 3 received an application of ground chalk (2 tons per acre), superphosphate of lime (5 cwt. per acre) and sulphate of potash (2 cwt. per acre), while during February, sub-plot 3 further received sulphate of ammonia at the rate of 2 cwt. per acre.

Table XVI. *Yield of hay and aftermath in* 1929 *and* 1930.

1929 Total meadow plot		1930					
		Sub-plot 1		Sub-plot 2		Sub-plot 3	
Hay (June 12) lb. dry matter per acre	Aftermath (Oct. 2) lb. dry matter per acre	Hay (June 10) lb. dry matter per acre	Aftermath (Oct. 3) lb. dry matter per acre	Hay (June 10) lb. dry matter per acre	Aftermath (Oct. 3) lb. dry matter per acre	Hay (June 10) lb. dry matter per acre	Aftermath (Oct. 3) lb. dry matter per acre
2590	140	3924	2196	3942	2202	4866	2556

Table XVII. *Composition of hay and aftermath cuts in* 1929 *and* 1930 *(dry matter basis)*.

Season ...	1929 Total meadow plot*		1930					
			Sub-plot 1†		Sub-plot 2‡		Sub-plot 3§	
	Hay %	After-math %	Hay %	After-math %	Hay %	After-math %	Hay %	After-math %
Crude protein	11·31	9·15	11·34	11·96	10·70	11·37	10·32	11·35
Ether extract	4·60	6·06	3·34	3·63	3·09	3·66	2·97	3·55
N-free extractives	51·06	51·94	47·43	46·80	47·19	48·48	46·19	46·77
Crude fibre	24·17	21·30	28·62	26·69	30·28	25·61	31·76	27·65
Ash	8·86	11·55	9·27	10·92	8·74	10·88	8·76	10·68
True protein	10·14	7·25	9·56	10·79	9·45	10·54	9·27	10·11
"Amides"	1·17	1·90	1·78	1·17	1·25	0·83	1·05	1·24
Lime	1·58	2·05	1·51	1·49	1·34	1·37	1·05	1·45
Phosphoric acid	0·64	0·66	0·79	0·85	0·75	0·84	0·72	0·85
Silica	1·86	5·11	1·84	4·58	1·82	5·33	2·24	5·19
Moisture as cut	77·70	54·60	79·90	78·50	80·10	76·00	80·90	75·10

* Lightly grazed by ewe flock during previous winter. No further fertilising.
† F.Y.M. plus chalk, potash and phosphate.
‡ F.Y.M. only.
§ F.Y.M. plus chalk, potash, phosphate and nitrogen.

Comments on Tables XVI and XVII.

The contrasting behaviour of the weather in the seasons of 1929 and 1930 is again brought out by the data in Table XVI for the yields of hay and aftermath from the meadow plot in these two seasons. The hay cut was very light in 1929, amounting to only 2590 lb. of dry matter per acre, compared with 3942 lb. from the meadow sub-plot 2 in 1930, while the growth of aftermath during the droughty second half of the 1929 season was almost negligible. The small amount of parched herbage obtained at the cutting of the aftermath on October 2 represented a yield of merely 140 lb. dry matter per acre.

The yield data for the differently treated meadow sub-plots in the favourable 1930 season present several noteworthy features. The hay yields from sub-plot 2 (farm yard manure only) and sub-plot 1 (farm yard manure plus chalk, potash and phosphate) are almost identical, being 3942 and 3924 lb. dry matter per acre respectively. The same statement also holds true of the aftermath yields of these sub-plots, namely, 2202 and 2196 lb. dry matter per acre respectively for sub-plots 2 and 1. It is clear, therefore, that the application of chalk, superphosphate of lime and sulphate of potash, in addition to farm yard manure, to the experimental plot produced no further improvement in the hay and aftermath crops than was effected by the application of farm yard manure alone. On the other hand, however, the stimulating effect of the application of sulphate of ammonia, in addition to farm yard manure, chalk, potash and phosphate, is very clearly evidenced by the yield data for the meadow sub-plot 3, the hay cut amounting to 4866 and the aftermath cut to 2556 lb. dry matter per acre (that is to say, 24 and 16·4 per cent. improvements over the corresponding yields from sub-plot 1). It would be unwise, however, to formulate any generalisations from the foregoing observations, since they may merely signify that the meadow plot was not in need of chalk, potash and phosphate, but was in a condition to respond to the application of a supply of easily available nitrogen.

Over the entire season of 1930, the total dry matter produced per acre from the meadow sub-plot 2, receiving farm yard manure only, was 52·5 per cent. greater than that obtained from the pasture sub-plot 11, which also received farm yard manure only and was cut every month. In contrast to this effect, however, it is interesting to note that the meadow sub-plot 3, receiving farm yard manure, chalk, potash, phosphate and nitrogen, produced only 28·8 per cent. more dry matter per acre over

the entire season than the pasture sub-plot 12, which was cut every month and received the same amounts of farm yard manure, chalk, potash and phosphate, but, instead of being dressed with 2 cwt. sulphate of ammonia in one application in February, had periodic small dressings, amounting in all to 3¾ cwt. per acre, throughout the season. It is to be concluded, therefore, that the periodic small dressings of sulphate of ammonia on the pasture sub-plot stimulated production to a greater degree than was manifested on the meadow sub-plot by the application of a single large dressing early in the year, it being kept in mind that both meadow and pasture sub-plots had also received, on the same dates, equal dressings of farm yard manure, superphosphate of lime, chalk and sulphate of potash, and further, that the total amount of sulphate of ammonia used on the pasture sub-plot was nearly twice as great, on the acre basis, as that applied to the meadow sub-plot.

In considering the data in Table XVII for the composition of the hay and aftermath cuts, the high percentage of lime in the parched herbage which formed the 1929 aftermath is noteworthy, a result in keeping with the finding, already noted, that the lime content of herbage rises during spells of droughty weather. It will also be noted that the hay and aftermath cuts in 1929 were poorer in crude fibre than the corresponding cuts in 1930, a fact which is brought out more fairly in Table XVIII, in which the percentages of crude protein and fibre are brought to the comparable basis of silica-free dry matter. This fact once more emphasises the conclusion which has been put forward in an earlier section, namely, that during a droughty season of retarded vegetative activity and growth, lignification of herbage sets in at an earlier stage of development, marked by a lower fibre content, than is the case in a moist season of quick and continuous growth.

Table XVIII. *Crude protein and fibre content of hay and aftermath in 1929 and 1930, on basis of silica-free dry matter.*

Season ...	1929 Total meadow plot		1930					
			Sub-plot 1		Sub-plot 2		Sub-plot 3	
	Hay %	After-math %	Hay %	After-math %	Hay %	After-math %	Hay %	After-math %
Crude protein	11·52	9·64	11·55	12·53	10·89	12·01	10·56	11·97
Crude fibre	24·63	22·45	29·16	27·97	30·84	27·05	32·49	29·16

An inspection of the data in Table XVIII brings out the fact that the 1929 aftermath contained a relatively low percentage of protein, a

further confirmation of the finding that the "browning" of herbage during droughty weather leads to a lowering of the protein content. The composition data for the hay and aftermath cuts from the three meadow sub-plots in 1930 have a special interest. In respect of the hay cuts, it will be seen that the produce from sub-plot 3 (complete fertilisers, including sulphate of ammonia) was poorest both in protein and lime, but richest in fibre, whereas the hay from sub-plot 1 (all fertilisers applied with the exception of sulphate of ammonia) was richest in protein and lime, but poorest in respect of fibre. The hay cut from sub-plot 2 (farm yard manure only applied) lay intermediate between the other samples in these respects.

At the time of mowing the meadow sub-plots, it was very apparent that wild white clover had displayed very vigorous growth on sub-plot 1 and was only moderately represented in the herbage of sub-plot 2. The main characteristics of the herbage on sub-plot 3, however, were the scarcity of wild white clover and its relative freedom from weeds. These observations, therefore, afford an adequate explanation of the differences respecting the protein and lime content of the hay cuts from the meadow sub-plots. It would appear that the application of chalk, potash and phosphate to sub-plot 1 had stimulated the development of the lime and protein-rich wild white clover, but that the additional application of sulphate of ammonia on sub-plot 3 had exerted a distinctly depressing influence on the growth of this species. In this case, the adverse effect could scarcely be attributed to direct contact action of sulphate of ammonia on the leaves of wild white clover, since the fertiliser was applied as early as February and was manifestly well washed into the soil before active growth of herbage had begun. Neither could it be attributed to the "smothering" of the clover by the grasses in the sub-plot, since, if this were the sole explanation, a similar effect should have been noted in the case of the herbage on sub-plot 1. It must therefore be concluded that the presence of sulphate of ammonia *in the soil* (or, alternatively, the products of its nitrification) can lead to a discouragement of wild white clover, although, of course, the other factors may operate in a contributory sense, especially if the fertiliser is applied after active growth has begun, as is the case when pastures are submitted to the modern system of intensive fertilising.

The relatively high fibre content of the hay from sub-plot 3, a feature which was also shared by the aftermath cut from the sub-plot, is an indication of the stimulation of vegetative activity, with consequent higher yield of herbage, brought about by the application of sulphate of

ammonia, lignification beginning under these conditions when the fibre content has reached a higher level than is the case when no artificial nitrogenous fertiliser is applied.

<center>SUMMARY.</center>

The object of this series of investigations is to secure detailed information concerning the composition, digestibility and nutritive value of pasture herbage in its different stages of growth. The results which were obtained in these respects by cutting the herbage of the experimental pasture plot at weekly, fortnightly and 3-weekly intervals have been described in previous communications. During the seasons of the present investigations (1929 and 1930), the trials have been carried a stage further by the adoption of a system of cutting at monthly intervals. The results, therefore, are invested with special significance, in that a period of four weeks has been tentatively adopted in this country as the interval which is allowed to lapse, in rotational grazing practice, between successive grazings of pasture enclosures.

The seasons of 1929 and 1930 were sharply contrasted in respect of weather, 1929 being a season of abnormally low rainfall, whereas 1930 was characterised by an abundance of well-distributed rainfall. No fertilisers were applied to the pasture plot during the season of 1929; the 1930 experiments, on the other hand, were carried out under conditions of intensive fertilising. The main findings of the investigations are recorded below.

1. *Chemical composition of monthly pasture cuts.* Pasture grass during April and early May contains, on the basis of dry matter, well over 20 per cent. of crude protein whether the system of cutting is weekly, fortnightly, 3-weekly or monthly; that is to say, its richness in protein, and indeed its general composition, both organic and inorganic, are, within the limits of the investigations so far carried out, independent of the frequency of the cuts. It is concluded, therefore, that when a pasture has been closely grazed in the previous autumn, there is little danger of the herbage running off in chemical composition during the April of the new season, even though the pasture is only lightly grazed during this month.

The distinction in composition between pasture herbage cut at weekly and at monthly intervals only becomes marked with the advent of the "flush" period of growth. At this stage of the 1929 season, for instance, the monthly cut grass contained 18·71 per cent. of crude protein and

18·84 per cent. of crude fibre (dry matter basis), while the weekly mown herbage contained 23·66 and 15·97 per cent. respectively of these constituents. Over the 1929 season (omitting the results for the droughty period of late August and September) the weekly cut herbage was some 3 per cent. richer than the monthly cut grass in respect of crude protein, slightly poorer in N-free extractives and significantly poorer in crude fibre. This constituted the main difference between the two types of herbage, the distinctions in respect of ether extract, lime and phosphoric acid being only slight. The mean crude protein content of the monthly cut grass, on the basis of dry matter, for the seasons of experiment was 20·23 per cent. (1929) and 19·35 per cent. (1930).

A comparison of the composition data for the two years of experiment leads to the conclusion that in a moist, quick-growing season, such as 1930, characterised by greatly enhanced vegetative activity in pasture plants, the amount of ether extract in the herbage of pastures may be depressed to a marked extent, and the crude protein to a slight extent, whereas the amount of crude fibre may be raised quite considerably.

It is concluded, on the basis of the 1930 results, that the use of fertilisers on an intensive scale, on this particular pasture and in this particular year, was not attended, during the first year of their application, by any important changes in the chemical composition of the herbage.

2. *Digestibility and nutritive value of monthly pasture cuts.* The conclusion is drawn that under a system of rotational grazing, the interval between successive close-grazings may be lengthened to a month and still the herbage during the early part of the season (April) will be equal in respect of digestibility, digestible protein content and starch equivalent to grass which is continuously and heavily grazed during this part of the year. This finding has an important application in grazing practice, in that it demonstrates that over-grazing of a pasture in the early part of the season is not necessary in order to ensure the best results from the standpoints of digestible protein content and nutritive value, provided, of course, that the herbage was grazed down efficiently at the close of the previous season. This statement holds true for both fertilised and unfertilised pastures. Herein lies a dual advantage, since (a) heavy grazing in the earliest part of the season may react adversely on the persistence of certain early grasses and on the productivity of the pasture in the later stages of the season, and (b) a more lenient system of grazing in early spring leads to a more abundant yield of herbage at that particular time of the year, especially if the weather conditions are not entirely favourable to the growth of pasture grass.

In the light of the results so far obtained in this series of investigations, the following general conclusion may be drawn. Pasture grass grown under a system of 3-weekly cuts is equal to weekly and fortnightly mown herbage in respect of digestibility. If the interval between successive cuts be lengthened to a month, the grass obtained in the early part of the season is as digestible as that obtained under systems of weekly, fortnightly and 3-weekly cutting. The effect of the longer interval on the digestibility of the herbage as the season advances will be determined largely by the weather conditions. If conditions are eminently favourable to quick growth, as in 1930, the digestibility of the herbage, including that of the fibre, will remain high, as under more severe systems of cutting, the only noticeable effect being a slight running-off in the digestibility of the protein constituent during the mid-season, followed by recovery at a later stage. If, on the other hand, the season is substantially one of drought, with consequent slow growth, the herbage will tend, as the season advances, to suffer some degree of lignification, and its digestibility will be lowered. In a season displaying more normal behaviour in respect of amount and distribution of rainfall, it is probable that the mid-seasonal falling-off in digestibility, followed by recovery in August and September, would not be very considerable, although probably more pronounced than would be experienced under a system of cutting every three weeks.

Dry mid-seasonal conditions have been found to give rise to a more pronounced depression of the digestibility of pasture herbage under a severe system of cutting than under a lenient system. There would appear to be, however, an optimum leniency of cutting, probably, so far as can be ascertained at present, when the interval between successive cuts is three weeks. If the interval be lengthened beyond this optimum period, the consequence may be an intensification of the depressing influence of the dry mid-season on the digestibility of the herbage such as is brought about by shortening the interval. It is hoped to secure further information on this point in the coming season (1931).

Lignification in herbage plants is apparently delayed until the final stages of fibre production. The process does not occur during the vegetative phase of development, but begins only in the late-flowering stage, or even during the period of seed formation, when the stems and leaves are being depleted of nutrients. If, however, persistent drought leads to an untimely check or cessation in the growth of herbage, then the lignification processes may set in at an earlier stage of distinctly lower fibre content than is indicated by the results of the quick-growing 1930 season.

The monthly pasture cuts of 1930 were as rich in total digestible organic matter as the grass obtained in previous years under less lenient systems of cutting, the percentage of digestible organic matter ranging from 69·98 to 75·57 per cent. of the dry matter, with a mean value of 72·47 per cent. Although during the April of the season there is no significant difference in respect of digestible protein content between grass submitted to cutting at weekly, fortnightly, 3-weekly and monthly cutting, differences become apparent as the season advances. In both 1929 (droughty season) and 1930 (plentiful rainfall), the digestible protein content of the herbage fell to lower levels under the system of monthly cuts than was the case in previous years when the plot was cut at shorter intervals. In both seasons, the lowest percentage of digestible protein was encountered in mid-June, shortly after the period of "zenith" growth on the pasture, being 13·45 per cent. in 1929 and 12·98 per cent. in 1930. Even as early as in May, however, the content of digestible protein in both years had fallen to a value lower than the minimum values for the previous seasons in which the influence of more frequent systems of cutting had been investigated. The averages of the digestible protein determinations over the entire season for the monthly pasture cuts were 14·76 and 14·70 per cent. of the dry matter for 1929 and 1930 respectively, the corresponding values for 1925 (weekly cutting), 1927 (fortnightly cutting) and 1928 (3-weekly cutting) being 19·97, 18·75 and 16·66 per cent. respectively. It is obvious, therefore, that as the system of cutting becomes more lenient, the percentage of digestible protein suffers a gradual and continuous decline.

The conclusion is drawn that in a season of ordinary rainfall, in respect both of amount and distribution, the falling-off in the starch equivalent of monthly mown herbage, as compared with herbage cut at more frequent intervals, would be slight and would be evidenced mainly in a somewhat greater mid-season range of depression. It is inferred that this conclusion would hold also for pastures submitted to rotational grazing with monthly intervals of unchecked growth, provided that at each grazing the herbage was efficiently and uniformly eaten down.

3. *Nutritive ratio of monthly pasture cuts.* The dry matter of monthly mown herbage during April contains nearly 20 per cent. of digestible protein and more than 70 per cent. of starch equivalent and is to be regarded as a protein-concentrated food. Obviously, at this stage, supplementary food must take the form of carbohydrate-rich feeding stuffs.

During May and June, however, under a monthly system of cutting, the nutritive ratio of the herbage widens considerably. Over this period,

which is important because it includes the "flush" period of growth, the 1930 herbage contained, on the basis of dry matter, about 13 per cent. of digestible protein and about 66½ per cent. of starch equivalent. The balance of the "concentrate" dry matter at this stage is much more suitable for general feeding purposes than was the case in previous years under more severe systems of cutting. A ration of such herbage containing 30 lb. of dry matter furnishes almost exactly the requirements of a 5-gallon cow in respect of starch equivalent and digestible protein. The question of the nutrition of dairy cows of varying yield on such pasturage is discussed in the text, and it is finally concluded that, beyond the limit of production of 5 gallons of milk per day, the concentrated food secured from rotationally-grazed pastures (monthly intervals) tends to become too bulky for the purpose, and that the dairyman in such cases should rely less and less on the grass, and more and more on the less bulky concentrated foods, as the yield of milk increases progressively beyond 5 gallons per day. For fattening stock on such pasturage, the correct form of supplement is the carbohydrate food.

The foregoing observations respecting the May and June herbage hold substantially for the herbage in July and August. During September, however, the herbage becomes distinctly richer again in digestible protein, and the need for carbohydrate supplements is consequently more obvious at this later stage.

4. *Yield results.* The main conclusion in this section may be stated as follows. Within the limits of the systems of cutting so far investigated (weekly to monthly intervals), monthly rotational close-grazing, reinforced by a suitable system of fertilising, provides the optimum conditions for the maximum yield of starch equivalent (*i.e.* of "keep") from pastures. Close-grazing at monthly intervals has the further advantage of conferring on the herbage a "balance" which renders it much more suited to form the sole diet of farm animals. The trend of the results indicates that if the interval between successive close-grazings is lengthened to five weeks, the herbage will be enabled to reach the stage of growth at which lignification sets in, with consequent depression of digestibility and nutritive value. A definite pronouncement on this point, however, must await the results of a further investigation to be carried out in 1931, when it is intended to examine the yield, composition and nutritive value of pasturage under a system of 5-weekly cuts.

It is important that the foregoing findings should be correlated with the observations recorded in the botanical section of this publication.

5. *Influence of prolonged drought on the composition and nutritive*

value of the monthly pasture cuts. The main effects of severe drought and the consequent "browning" of pasture herbage are: (*a*) a very decided falling-off in the percentage of protein; (*b*) a slight increase in the percentages of N-free extractives and crude fibre; (*c*) an abrupt rise in the percentage of lime, accompanied by a decline in the percentage of phosphoric acid; (*d*) a pronounced reduction of the moisture content of the herbage; (*e*) a decided diminution in digestibility and nutritive value, attributable to the cessation of vegetative activity and the premature setting-in of lignification. These changes are simply an accentuation, particularly in respect of protein content and digestibility, of the known temporary effects which are produced in herbage during mid-season in consequence of the dry weather which is usually encountered at that time of the year.

6. *Yield and composition of hay and aftermath.* The results of investigations into the composition and yield of hay and aftermath in the seasons of 1929 and 1930, with and without application of fertilisers, are brought forward and discussed in the final section of the paper, together with a tentative discussion as to the mode of action of sulphate of ammonia in depressing the development of wild white clover on meadows and pastures.

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APPENDIX. *Digestion tables.*

Season 1929.

Sheep XI

	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.
Period 1							
Daily ration	1043·60	934·44	230·32	57·61	512·30	134·21	109·16
Food residues	32·80	18·90	4·66	1·17	10·38	2·71	13·90
Net consumption	1010·80	915·54	225·66	56·44	501·94	131·50	95·26
Voided	223·50	158·39	43·74	20·58	64·70	29·37	65·11
Digested	787·30	757·15	181·92	35·86	437·24	102·13	30·15
Digestion coefficients (%)	77·89	82·70	80·62	63·54	87·11	77·67	31·65
Period 2							
Daily ration	930·60	828·79	174·49	71·10	427·52	155·68	101·81
Food residues	20·20	14·30	3·01	1·23	7·38	2·68	5·90
Net consumption	910·40	814·49	171·48	69·87	420·14	153·00	95·91
Voided	211·80	163·64	42·34	20·14	65·87	35·29	48·16
Digested	698·60	650·85	129·14	49·73	354·27	117·71	47·75
Digestion coefficients (%)	76·74	79·91	75·31	71·18	84·32	76·93	49·78
Period 3							
Daily ration	1038·80	933·67	186·67	69·49	483·36	194·15	105·13
Food residues	17·30	11·30	2·26	0·84	5·85	2·35	6·00
Net consumption	1021·50	922·37	184·41	68·65	477·51	191·80	99·13
Voided	272·30	212·50	45·99	27·88	88·99	49·64	59·80
Digested	749·20	709·87	138·42	40·77	388·52	142·16	39·33
Digestion coefficients (%)	73·34	76·96	75·06	59·39	81·37	74·12	39·68
Period 4							
Daily ration	1319·00	1179·71	247·05	76·50	613·86	242·30	139·29
Food residues	18·80	12·96	2·71	0·84	6·75	2·66	5·84
Net consumption	1300·20	1166·75	244·34	75·66	607·11	239·64	133·45
Voided	426·30	337·20	62·58	47·70	151·04	75·88	89·10
Digested	873·90	829·55	181·76	27·96	456·07	163·76	44·35
Digestion coefficients (%)	67·21	71·10	74·39	36·96	75·12	68·34	33·23
Period 5							
Daily ration	939·90	847·97	176·04	62·69	437·99	171·25	91·93
Food residues	13·00	8·20	1·70	0·61	4·24	1·65	4·80
Net consumption	926·90	839·77	174·34	62·08	433·75	169·60	87·13
Voided	294·70	233·93	46·71	18·45	101·29	51·48	60·77
Digested	632·20	605·84	127·63	27·63	332·46	118·12	26·36
Digestion coefficients (%)	68·21	72·14	73·21	44·51	76·65	69·65	30·25

Sheep XII

	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.
Period 1							
Daily ration	1205·60	1079·50	266·08	66·55	591·83	155·04	126·10
Food residues	14·20	5·10	1·26	0·31	2·80	0·73	9·10
Net consumption	1191·40	1074·40	264·82	66·24	589·03	154·31	117·00
Voided	271·30	188·34	58·00	25·80	69·92	34·62	82·96
Digested	920·10	886·06	206·82	40·44	519·11	119·69	34·04
Digestion coefficients (%)	77·23	82·47	78·10	61·05	88·13	77·57	29·10
Period 2							
Daily ration	930·60	828·79	174·49	71·10	427·52	155·68	101·81
Food residues	2·90	1·70	0·36	0·15	0·88	0·31	1·20
Net consumption	927·70	827·09	174·13	70·95	426·64	155·37	100·61
Voided	227·40	176·15	45·21	20·60	71·27	39·07	51·25
Digested	700·30	650·94	128·92	50·35	355·37	116·30	49·36
Digestion coefficients (%)	75·49	78·70	74·04	70·97	83·29	74·85	49·06
Period 3							
Daily ration	1038·80	933·67	186·67	69·49	483·36	194·15	105·13
Food residues	3·70	1·60	0·32	0·12	0·83	0·33	2·10
Net consumption	1035·10	932·07	186·35	69·37	482·53	193·82	103·03
Voided	288·80	221·51	51·18	30·01	92·55	47·77	67·29
Digested	746·30	710·56	135·17	39·36	389·98	146·05	35·74
Digestion coefficients (%)	72·10	76·24	72·54	56·74	80·82	75·35	34·69
Period 4							
Daily ration	1319·00	1179·71	247·05	76·50	613·86	242·30	139·29
Food residues	4·00	1·24	0·23	0·08	0·65	0·25	2·76
Net consumption	1315·00	1178·47	246·79	76·42	613·21	242·05	136·53
Voided	424·10	333·69	65·40	50·34	147·04	70·91	90·41
Digested	890·90	844·78	181·39	26·08	466·17	171·14	46·12
Digestion coefficients (%)	67·75	71·69	73·50	34·13	76·02	70·70	33·78
Period 5							
Daily ration	939·90	847·97	176·04	62·69	437·99	171·25	91·93
Food residues	4·30	2·30	0·48	0·17	1·19	0·46	2·00
Net consumption	935·60	845·67	175·56	62·52	436·80	170·79	89·93
Voided	295·40	233·46	43·63	33·71	107·67	48·45	61·94
Digested	640·20	612·21	131·93	28·81	329·13	122·34	27·99
Digestion coefficients (%)	68·43	72·39	75·15	46·08	75·33	71·63	31·12

NOTE. The care of the experimental animals throughout the trials was in the hands of Messrs V. Thurlbourn and C. Bendall.

APPENDIX. *Digestion tables (contd).*

Season 1930.

	Sheep XIII							Sheep XIV						
	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.
Period 1														
Daily ration	876·40	785·34	203·94	29·71	398·06	153·63	91·06	875·90	784·89	203·82	29·69	397·83	153·55	91·01
Voided	168·10	122·14	34·21	16·42	47·62	23·89	45·96	185·20	138·14	38·84	17·85	55·39	26·06	47·06
Digested	708·30	663·20	169·73	13·29	350·44	129·74	45·10	690·70	646·75	164·98	11·84	342·44	127·49	43·95
Digestion coefficients (%)	80·82	84·45	83·23	44·73	88·04	84·45	49·53	78·85	82·40	80·94	40·00	86·07	83·03	48·29
Period 2														
Daily ration	812·00	724·79	137·23	33·37	367·92	186·27	87·21	812·00	724·79	137·23	33·37	367·92	186·27	87·21
Food residues	6·20	3·40	0·64	0·16	1·73	0·87	2·80	2·70	0·60	0·11	0·03	0·31	0·15	2·10
Net consumption	805·80	721·39	136·59	33·21	366·19	185·40	84·41	809·30	724·19	137·12	33·34	367·61	186·12	85·11
Voided	188·60	143·22	33·91	15·01	65·93	28·37	45·38	177·00	135·16	31·74	14·34	61·27	27·81	41·84
Digested	617·20	578·17	102·68	18·20	300·26	157·03	39·03	632·30	589·03	105·38	19·00	306·34	158·31	43·27
Digestion coefficients (%)	76·59	80·15	75·18	54·80	82·00	84·70	46·24	78·13	81·34	76·85	57·00	83·33	85·06	50·84
Period 3														
Daily ration	929·20	814·54	154·25	36·61	406·25	217·43	114·66	929·20	814·54	154·25	36·61	406·25	217·43	114·66
Food residues	9·50	5·70	1·08	0·26	2·84	1·52	3·80	3·20	0·60	0·11	0·03	0·30	0·16	2·60
Net consumption	919·70	809·84	153·17	36·35	403·41	215·91	110·86	926·00	813·94	154·14	36·58	405·95	217·27	112·06
Voided	243·20	189·67	39·88	19·75	89·25	40·79	53·53	223·90	173·72	35·82	17·62	83·89	36·39	50·18
Digested	676·50	619·17	113·29	16·60	314·16	175·12	57·33	702·10	640·22	118·32	18·96	322·06	180·88	61·88
Digestion coefficients (%)	73·56	76·55	73·90	45·67	77·88	81·11	51·71	75·82	78·66	76·76	51·83	79·34	83·25	55·22
Period 4														
Daily ration	1289·60	1137·18	216·72	48·75	610·30	261·41	132·42	1269·60	1137·18	216·72	48·75	610·30	261·41	132·42
Voided	336·60	266·08	53·69	33·35	123·06	55·98	70·52	335·20	262·83	50·85	31·78	124·46	55·74	72·37
Digested	933·00	871·10	163·03	15·40	487·24	205·43	61·90	934·40	874·35	165·87	16·97	485·84	205·67	60·05
Digestion coefficients (%)	73·49	76·60	75·23	31·59	79·84	78·59	46·75	73·60	76·89	76·54	34·81	79·61	78·68	45·35
Period 5														
Daily ration	862·40	763·57	152·90	36·31	393·17	181·19	98·83	862·40	763·57	152·90	36·31	393·17	181·19	98·83
Voided	206·80	152·16	34·97	20·60	64·87	31·72	54·64	205·80	148·05	33·36	19·70	64·86	30·13	57·75
Digested	655·60	611·41	117·93	15·71	328·30	149·47	44·19	656·60	615·52	119·54	16·61	328·31	151·06	41·08
Digestion coefficients (%)	76·02	80·07	77·13	43·27	83·50	82·49	44·71	76·14	80·61	78·18	45·74	83·50	83·37	41·57
Period 6														
Daily ration	936·00	832·76	178·50	43·99	424·57	185·70	103·24	936·00	832·76	178·50	43·99	424·57	185·70	103·24
Voided	232·60	177·17	38·52	27·38	75·92	35·35	55·43	227·10	169·50	34·61	28·09	72·49	34·31	57·60
Digested	703·40	655·59	139·98	16·61	348·65	150·35	47·81	708·90	663·26	143·89	15·90	352·08	151·39	45·64
Digestion coefficients (%)	75·15	78·72	78·42	37·76	82·12	80·96	46·31	75·74	79·65	80·61	36·15	82·93	81·52	44·21

THE STICKY POINT WATER OF SOILS.
PART II[1].

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(With Three Text-figures.)

I.

KEEN and COUTTS(8) and Coutts(1) have suggested that the percentage of water in a soil at the sticky point is related to the colloidal content of the soil, as measured by "loss on ignition," in the following manner:

$$S = mI + c,$$

i.e. by a rectilinear equation, and point out that the figure obtained for c approximates to the theoretical percentage of water held in the interstitial spaces of an ideal soil.

Coutts(1) and the writer(10) have pointed out that sticky point water and water capacity as determined by the Keen-Raczkowski box experiment(7) are closely related to one another.

Pore space (p) determined by the box experiment is essentially a measure of the water capacity (w); it is an expression by volume of the wet soil while the water capacity is an expression by weight of the dry soil, but both express the same thing. Strictly speaking this is only true if water capacity be calculated on the basis of the soil left in the box after scraping off the expanded portion. If this latter value (w_1) be taken for the water capacity, it is related to pore space by

$$w_1 = \frac{100p}{(100 - p)\, s_1}.$$

While w_1 is not equal to w, for practical purposes the latter may be regarded as the weight percentage corresponding to the volume percentage p.

Sticky point water expressed as a percentage by weight of the dry soil is related to sticky point water by volume of the wet soil by a similar expression.

$$S = \frac{100Sv}{(100 - Sv)\, s}.$$

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[1] Part I, *South African J. Sci.* (1930), **27**, 183.

In these expressions s and s_1 are the specific gravities of the soil under the respective experimental conditions.

Now we have shown, and our results have been confirmed by Coutts, that pore space is related to the clay content by a rectilinear equation, so that it seems reasonable to assume that sticky point water by volume may also be related to the clay content by a similar expression. On the other hand, Keen and Coutts and Coutts consider "loss on ignition" to be a better measure of the colloid content of the soil than the percentage of clay, so, though our own results and those of the Sudan laboratory (16) do not confirm this finding, it is better to study the question of the relation between sticky point water and colloid content by methods which are independent of any assumption as to whether loss on ignition or clay content is the better measure of the colloid status of the soil.

We may regard pore space as the amount of water held by the colloidal portion of the soil as imbibed water plus the volume required to fill the interstitial spaces under the experimental conditions. Similarly, sticky point water may be regarded as the colloidally imbibed water plus the volume in the interstitial spaces, the latter volume differing from that in the case of the pore space owing to the difference in the degree of packing. The actual amount of water imbibed by the colloid should be the same in both cases. Now if, following Keen and Coutts, we regard the soil at the sticky point as being approximately packed in the closest possible manner, a not unreasonable assumption in view of the thorough kneading to which the wet soil is subjected during the course of the determination, it would seem that the volume of water occupying the interstitial spaces should approximate to the value for an ideal soil in closest packing. Using the percentage of clay as a measure of the colloid content, we were able to show that this is the case (10).

If sticky point water by volume can be expressed in terms of the colloid content of the soil in the manner indicated above, namely, that it is equal to the water imbibed by the colloid plus a constant volume of water which fills the interstitial spaces, and if we assume that each unit of colloid imbibes a definite quantity of water, we arrive at an expression of the type

$$Sv = m \text{ (colloid)} + K,$$

where m is a factor representing the weight of water imbibed by each percentage of colloid in the soil, and K is a constant approximating to the interstitial space in an ideal soil.

Since sticky point water by weight is related to sticky point water by volume, thus

$$S = \frac{100 Sv}{(100 - Sv)\, s_1};$$

if sticky point water by volume is related to the colloid content by a rectilinear equation, it is obvious that the relation between sticky point water by weight and colloid content must be represented by a curve of the type

$$ax - by - cxy = d.$$

Similarly if sticky point water by weight is related to the colloid content by a rectilinear expression, that between colloid and sticky point water by volume must also be represented by a curve.
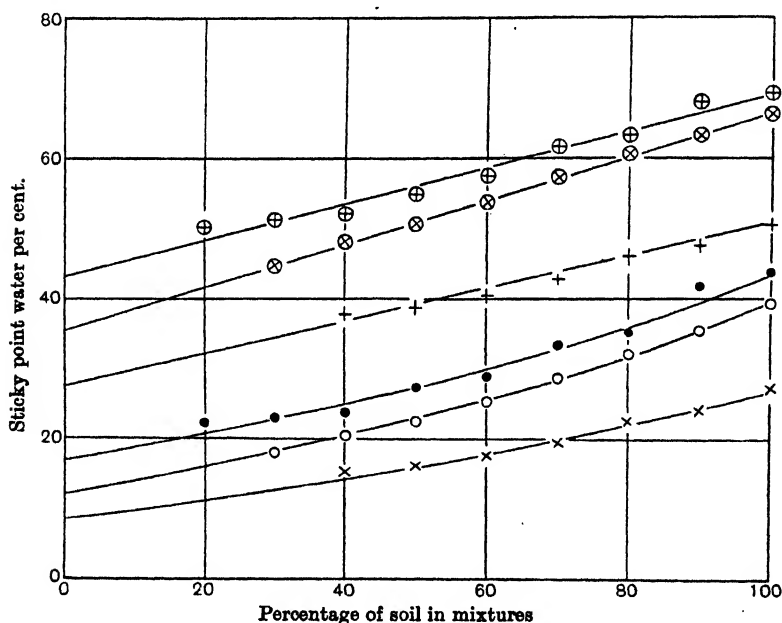
Fig. 1.

× Soil R.T., percentage by weight.   ⊗ Clay H, percentage by volume.
+ Soil R.T., percentage by volume.   ● Soil B.T., percentage by weight.
O Clay H, percentage by weight.   ⊕ Soil B.T., percentage by volume.

It is, however, not impossible that the relations between amount of colloid and sticky point water by weight and between amount of colloid and sticky point water by volume may both be curvilinear, though both

cannot be rectilinear. In order to test this point the following experiments were made.

1. Determinations of the percentage of sticky point water by weight were made on mixtures of soil and sand containing varying proportions of soil and, therefore, of colloid. As the same soil is used in each series, the results are not affected by the capacity of the soil colloid to imbibe water, that is, m should be constant for each series. There are no doubt variations in K, due to the replacement of swollen colloidal particles by sand, particles which do not occupy exactly the same space. The specific gravity of such mixtures is reasonably constant, so that variations in the relationship between S and Sv due to variations in specific gravity are negligible. Two samples of soil and one of a natural "pot-clay" were used. From the weight percentages the volume percentages were calculated, using the specific gravities found for the pure soil by the volumetric sticky point method in the formula given on p. 324.

The data are plotted in Fig. 1. In order to separate the curves the following adjustments have been made:

Weight percentage:	Curve R.T.	Actual
	Curve H.	Actual + 5 %
	Curve B.T.	Actual + 5
Volume percentage:	Curve R.T.	Actual + 10
	Curve H.	Actual + 20
	Curve B.T.	Actual + 20

2. Three series of similar determinations of both weight and volume percentages were made on:

(1) A sample of soil, 56/3v.
(2) A sample of natural "pot-clay," C.F.
(3) A sample of highly colloidal soil material prepared by removing most of the coarse sand and fine sand from the colloidal soil, B.T. This is marked sample A.

The results are plotted in Figs. 2 and 3.

The straight lines were calculated by the method of least squares and the curves obtained from those lines by the expression

$$S = \frac{100 Sv}{(100 - Sv) s_1},$$

the mean specific gravity found by the volume sticky point method being used in each case.

The curves were tested for "goodness of fit" by the χ^2 method given by Fisher[2]. For the two samples of soil and one of clay on which only

determinations of the sticky point water by weight were made, the procedure was to calculate the volume percentages from the weight percentages by using the specific gravity found for the pure soil. Our other experiments indicate that this value is representative of the average for all mixtures of a particular soil and sand. A line was found for these points by the method of least squares, and from points on this line values for sticky point water by weight were re-calculated, using the specific gravity as before. Straight lines were found by the method of least squares for the weight percentage points on the assumption that per-
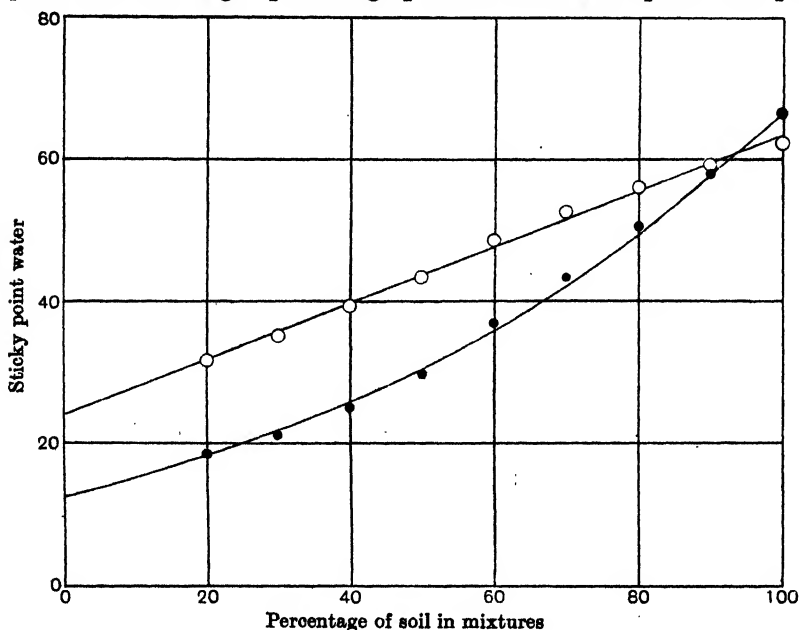
Fig. 2.

Soil A: percentage by weight, ●.   Soil A: percentage by volume, ○.

centage by weight of water at the sticky point is a rectilinear function of the amount of soil, and χ^2 for each case calculated from these lines. The value of χ^2 was also calculated for the curves obtained for soil-sticky point by weight. All these values are given in Table I.

It is apparent that, except in the case of the clay C.F., the curves fit the data for sticky point water by weight better than the straight lines. For the clay C.F. both series of points may possibly lie on curves, though the volume percentages undoubtedly lie more nearly on a straight line than do the weight percentages.

Table I.

Sample	Sv Straight line	S Straight line	Curve
Soil B.T.	—	3·4580	0·5160
Soil R.T.	—	0·1869	0·1170
Clay H	—	0·1909	0·0162
Clay C.F.	0·1561	0·4889	0·4745
Soil 56/3v	0·0193	0·1654	0·0345
Soil A	0·0802	1·9199	0·2139

The heading "Values of χ^2" spans the Sv and S columns; "S" spans the Straight line and Curve columns.
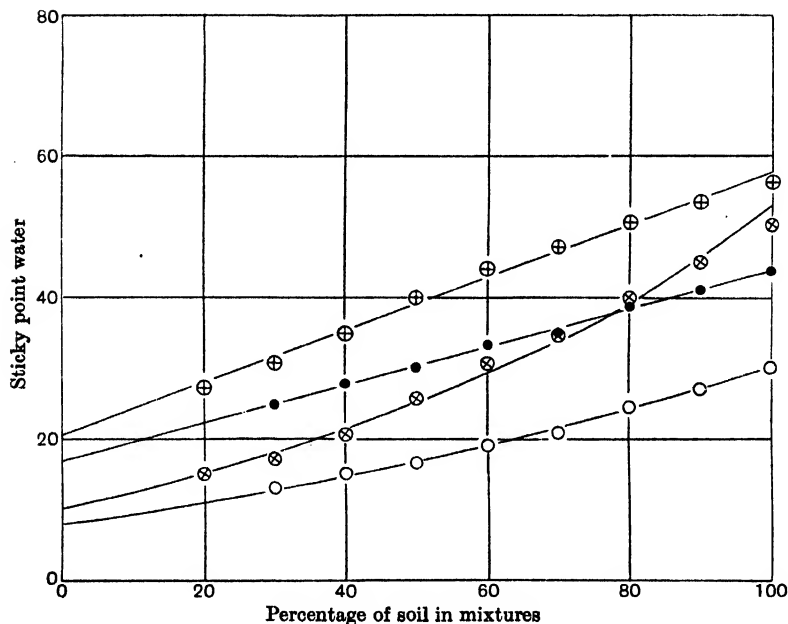
Fig. 3.

○ Soil 56/3v, percentage by weight.   ⊗ Clay C.F., percentage by weight.
● Soil 56/3v, percentage by volume.   ⊕ Clay C.F., percentage by volume.

In the case of the colloidal soil A there can be no doubt that the weight percentages lie on a curve, while the volume percentages are well represented by a straight line. Taking the data as a whole, they may be regarded as furnishing good confirmation of the view that the volume percentage of water at the sticky point is a rectilinear function of the percentage of colloid and that the weight percentage is not.

For a specific gravity of 2·5, which is the approximate value for the soil-sand mixtures holding that amount of water at the sticky point,

$$S = Sv,$$

when $$(100 - Sv)\, s = 100,$$

i.e. when $$Sv = 60.$$

It is interesting to note that the experimental points require that, in the case of the colloidal soil A, the two lines should cut at about this point, and also that beyond this point S is greater than Sv, while below it Sv is greater than S.

In practice no great error is made if it is assumed that S is a rectilinear function of the colloid content, provided the relationship is considered to hold over a limited portion of the curve and that no attempt is made at extrapolation. Table II gives for volume and weight percentages the extrapolated values for the sticky point water in soils containing no colloid.

Table II.

Sample	Sv_0 Straight line	S_0 Straight line	Curve
Soil B.T.	23·1	4·8	12·0
Soil R.T.	17·6	6·4	8·4
Clay H	15·5	3·0	8·7
Clay C.F.	20·4	3·8	10·0
Soil 56/3v	16·8	5·0	7·9
Soil A	24·0	2·2	12·4

In how far these figures are affected by the grade of sand used it is impossible to say.

Determinations were made of the sticky points of mixtures of equal parts of a highly colloidal soil B, and seven different samples of sand varying widely in size and shape of particle. If the value of K or Sv_0 were independent of the type of sand used, it should be possible to determine this value, and also the slope of the line by determining Sv for the soil alone and for a mixture of, say, half sand and half soil. In order to get some idea of the variations in the values of the constants introduced by varying the type of sand, determinations were made of S and Sv for mixtures of equal parts of the soil B.T. and six samples of sand. The sands ranged in fineness from largely find sand, No. 7, to a coarse heterogeneous sea-sand, No. 1, containing a large proportion of platey shell fragments. The results of these two series of experiments are given in Table III. The values for soil B are single determinations, those for

soil B.T. are the means of duplicates which agreed well. In the case of B.T. the values for the constants have been calculated for each sand and are also given in the table.

Table III. *Effects of various kinds of sand.*

	Value determined	Sand no.						
		1	2	3	4	5	6	7
Soil B	S	26·1	27·7	27·4	28·4	26·3	25·7	27·9
Soil B.T.	S	21·3	21·1	24·6	21·3	22·4	—	24·3
	Sv	35·5	35·1	38·5	35·3	36·5	—	38·6
	Constant m	0·284	0·292	0·224	0·288	0·264	—	0·222
	Constant K	21·3	20·5	27·3	20·9	23·3	—	27·5

Though wide differences in the grade and heterogeneity of the sand affected the results comparatively little, the differences not being sufficiently greater than the possible standard deviation, a method for the estimation of the constants for any particular soil would require closer agreement. It would appear also by analogy that variations in the amount of silt would also materially affect the constants. The values in Table II must be viewed in this light and regarded as comparative. It is of interest to note that the line given by the data for soil B.T. and sand No. 5, namely, $Sv = 0·264$ (soil) $+ 23·3$, is practically the same as that calculated from the sticky point weight percentage data, for which the same sand was used, shown in Fig. 1, namely, $Sv = 0·259$ (soil) $+ 23·1$.

II. EFFECT OF ELECTROLYTES IN SOIL.

It is of especial importance in a country in which saline soils are of common occurrence to know how, and to what extent, the determinations of physical properties are affected by the salts present. In the present case of sticky point water, the question arises whether this figure represents the ultimate texture or degree of colloidality, or the structure or state of the colloidal material. In our own part of the country, the south-western or so-called Western Province, salinity is not a factor of great importance and highly saline soils do not occur to any extent. It was impossible, therefore, owing to lack of suitable material, to experiment on saline soils. In order, however, to throw some light on the question, sticky point determinations were made on a few of the more clayey of the soils dealt with in the former paper[10], after the soils had been wetted with solutions of sodium chloride or sodium carbonate instead of water, the determination being then made in the usual way. Correction was made for the amount of salt added on the basis of the

water content at the sticky point. This is not strictly correct, as the actual amount of solution added is greater than is represented by the water remaining at the sticky point. The discrepancy is, however, so slight that the results are not affected as was shown by determinations of the actual amounts of sodium chloride left in the dry soil in the experiments where the more concentrated solutions were used.

The results are set out in Table IV. The figures in the second column are the average values for sticky point water together with their standard deviations.

Table IV.

Soil no.	Water	Sticky point (S)				
		$N/100$ NaCl	$N/10$ NaCl	N NaCl	$N/10$ Na$_2$CO$_3$	$N/5$ Na$_2$CO$_3$
162	22·4±0·7	22·4	22·0	21·3	17·2	15·2
163	23·0±0·4	22·1	22·1	20·6	19·7	16·8
164	26·2±0·3	26·8	25·3	21·7	23·3	19·8
117	20·1±0·4	19·9	20·1	19·2	17·2	16·0
878	22·7±0·5	22·6	21·2	21·2	20·4	20·0
888	20·4±0·5	20·3	19·4	18·3	20·1	18·8
B.T.	38·8±1·6	38·9	39·8	39·6	37·7	38·4
R.T.	27·4±0·9	28·6	27·6	26·9	25·4	24·3

The results are anomalous in some respects. It is evident that sodium chloride in concentration up to $N/10$ or, for a sticky point of 20 per cent., up to about 0·12 per cent. of soil, has no effect on the sticky point. In concentration N, or 1·2 per cent. of soil for 20 per cent. sticky point water, the effect is in some cases to reduce the percentage of water at the sticky point, though, except for sample No. 164, the decrease is not very great. The determination in the case of this sample was done in triplicate, the three results being in close agreement so that the marked decrease in this case is not fortuitous.

Sodium carbonate definitely reduces the percentage of water at the sticky point except in the highly colloidal soil B.T., the value for which is not affected by either sodium chloride or sodium carbonate in the concentrations used. The results are of interest when considered in conjunction with the experiments of van Wyk[14] and of Oakley[13]. These workers found that the higher the concentration of the salt used the lower is the figure obtained for water absorbed. It is, of course, possible that the amount of water held in the interstitial spaces as well as that absorbed by the colloid may be influenced by the concentration of salts.

It seems probable that the sticky point of saline soils will indicate the state of the colloidal material rather than its amount, though much

further work especially on naturally saline soils is necessary to clear up the matter. It would seem, however, that in order to bring a soil containing sodium carbonate to the sticky condition, less water is required than would be necessary to bring a similar but alkali-free soil to an objectionably sticky state. If this is so, its bearing on the puddling of "black alkali" soils may be important.

In this connection the effect due to possible removal of salts by the hydrogen peroxide treatment, employed by Keen and Coutts to eliminate the effect of organic matter, must not be lost sight of. Of the samples examined by these workers, four, a raw subsoil, a commercial purified kaolin, and two soils gave higher sticky points after peroxide treatment. These two soils came from the Sudan and from Palestine. Of the soils examined in regard to "single value" properties at Khartoum[16], four of the five Sudan samples gave higher values for sticky point after peroxide treatment, the fifth value being the same in treated as in untreated soil. The four soils are described as "heavy alkaline soils." It is at least a possibility that the increase in the values for sticky point water, in spite of the elimination of one of the factors on which those values are supposed to depend, may be due to the removal of salts.

III. LOSS ON IGNITION AS AN INDEX OF COLLOIDAL STATUS.

The view expressed in the previous paper[10] that, for Transvaal and Cape Western Province soils, loss on ignition is not the good index of colloidal status that Keen and Coutts[8] and Coutts[1] have found it to be for European and Natal soils, is confirmed by those expressed in the *Report*, since received, of the Government Chemist, Khartoum, Sudan, for 1929[16]. It appears that humified organic matter as commonly found in European soils exerts a profound influence on the physical properties of soils, so much so that the influence of the mineral colloidal material is overshadowed or at least masked to a large extent. In countries where the humus content of the soils is low the converse is apparently true. It is interesting to recall that the bearing of composition of the clay on colloidal properties first received attention, independently and at about the same time, outside Europe from Hardy[3] in the West Indies, Joseph and Hancock[5] in the Sudan and the writer[9, 12] in the Transvaal. The reason for this is undoubtedly because, owing to the relatively small part played by organic colloids, attention was focussed on the inorganic colloids resulting in the adoption of the silica-alumina or silica-sesquioxide ratio as an index of the degree to

which colloidal properties are developed in the clay complex. The fact that of two large groups of heavy soils occurring in the Transvaal, a red and a black which have been described in previous papers (9, 11, 12), the average loss on ignition is as high, if not slightly higher, in the former than in the latter group, while the latter exhibits much more pronounced colloidal properties, shows that loss on ignition is not a satisfactory measure of colloidal status. The decided difference in colloidal properties found for these two groups of soil led to the conclusion that there must be some fundamental difference in the nature of the inorganic colloid. Measured by loss on ignition the red soils with rather less clay should have colloidal properties to at least the same degree as the black, whereas, in fact, black soils with far less clay and lower loss on ignition have greater plasticity, greater affinity for water, greater swelling and in general properties of this type to a greater degree. The explanation of this difference between British soils and soils from the Sudan and Transvaal is probably due to lack in the latter of easily decomposed organic matter having colloidal properties, loss on ignition being a measure chiefly of combined water and organised organic matter. As Whitfeild has pointed out, in the Sudan *Report* already referred to, this water of constitution is probably dependent on the quantity and chemical nature of the clay complex and not on its physical properties. Per unit of clay the type high in sesquioxides found in the red Transvaal soils appears to contain more combined water than the siliceous type occurring in the black soils. It is interesting to note also in this connection that Joseph and Snow (6) and van Zyl (15) found that hydrogen peroxide pre-treatment is unnecessary in the mechanical analysis of Sudan and South African soils. This is apparently because humified organic matter, the removal of which is the object of the treatment, is practically absent from these soils.

Another and even more weighty objection to the use of the "loss on ignition" as an index of colloid status is the fact that some ignited soils and clays have a perfectly definite sticky point, and that this may be higher than any possible value for water held in the interstitial space. The sample of clay, C.F., actually held, in most of our experiments, more water at the sticky point after ignition than it did before. The value depends on the intensity of the ignition, and cannot be reproduced with certainty, though the figures for loss on ignition do not vary appreciably. The sticky point figure appears to depend on the degree to which the particles of clay are sintered together and is lower when the portion taken for ignition is lumpy. The following figures (Table V) were obtained

for this sample which, it may be said, is a naturally occurring subsoil clay not treated in any way except by crushing.

Table V.

| | | Sticky point water | | |
No.		Weight percentage S	Volume percentage Sv	Loss on ignition
	Not ignited	50·2*	56·3*	—
1	Ignited	59·8	—	6·00
2	,,	51·9	—	—
3	,,	53·2	—	6·30
4	,,	46·8	55·5	6·43
5	,,	49·2	58·9	6·42
6	,,	53·5	63·4	6·23

* Mean of three determinations.

No. 1 was the residue from a determination of loss on ignition made by an assistant, the ignition having been carried out over a teclu burner. Nos. 4 and 5 were ignited to bright redness in a muffle furnace, while Nos. 2, 3 and 6 were ignited over a No. 4 Meker burner. The loss on ignition consists almost entirely of combined water. This combined water is not taken up again during kneading as was shown by determinations on the dry residue after sticky point determinations.

While in only one other case, also a clay, was the sticky point water more after ignition than before, several soil samples were found to show decided stickiness after ignition. For example, the soil B.T. gave $S = 32·1$ and $Sv = 40·0$ after ignition. It seems that the property of plasticity is not necessarily lost on ignition, and for this reason alone "loss on ignition" is not a satisfactory criterion of colloidal status. Our results and those of the Sudan laboratory show that it is equally unsatisfactory for other reasons.

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A COMPARISON OF METHODS FOR DETERMINING THE HYDROGEN-ION CONCENTRATION OF SOILS.

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(With Six Text-figures.)

DURING the past few years the systematic examination of an increasing range of Australian soils has resulted in the accumulation of much information regarding their pH values. The first comprehensive list was that published in 1927 by Prescott[29] for South Australian soils, and since then further data have been accumulating. The quinhydrone electrode has been used almost exclusively, and on account of the increasing importance of the work and the high pH values recorded in many cases, it has been considered necessary to standardise the various methods available. Although Australian soils exclusively have been investigated, the results obtained are sufficiently wide in their application to be of interest to workers elsewhere. A number of soils representative of the major types were chosen, and their pH values determined by the quinhydrone and hydrogen electrodes. When it became clear that the two methods gave divergent values in certain types of soil, the pH values were also determined by means of the antimony oxide electrode.

The choice of methods used was based on the following considerations. In solutions of known composition and in the absence of disturbing substances, the hydrogen electrode is the standard with which all other methods are compared. The hydrogen electrode will give an absolute measure of the hydrogen-ion concentration or activity[1] of a solution only when poisons and substances which react with hydrogen in the presence of platinum black are absent. In general, traces of the latter class of substances, such as dissolved oxygen, nitrates, Fe^{+++} and the like, do not cause a "depolarisation" of the electrode, as it is so quick acting and reversible that it is able to deal with small quantities of these[10, 19]. Larger quantities, however, either cause the electrode to lag in the attainment of the true equilibrium, although steady and

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[1] The difference between ionic concentrations and activities in such dilute solutions as are met with in soils would fall well within the experimental error of the determinations, and for convenience the term "concentration" is used throughout.

reproducible values may still be obtained, or they may cause the electrode to give fluctuating and altogether false values, depending on the nature of the disturbing reaction and concentration of the reactive substances. On account of the complexity of soil suspensions and the presence in them of dissolved oxygen and other reducible substances, it does not seem probable that the hydrogen electrode will give absolute values. However, on account of its rapid adaptability and the low concentration of such substances in soils, it may be expected to give values for the majority of soil suspensions which will differ from the true value by less than the experimental error involved in the determination. In consequence, the hydrogen electrode has been adopted as a standard for soils. It does not follow that it is to be regarded as the standard for all soils. When dealing with solutions of comparatively unknown composition it becomes necessary to compare values obtained by two or more methods, preferably subject to different limitations. As a result of such comparisons the methods which are most likely to give the correct value for any soil type can be determined.

The limitations of the quinhydrone electrode for general work are not so serious as those of the hydrogen electrode, and, in general, substances which interfere with the smooth operation of the quinhydrone electrode have an even greater disturbing effect on the hydrogen electrode. The number of cases in which the quinhydrone electrode has been used with satisfactory results, particularly in the presence of oxidising agents, after the hydrogen electrode has been proved inapplicable, is too great to list in this paper, and it was because of this and its greater simplicity that the quinhydrone electrode was adopted for soil work.

Although little is known of the limitations of the antimony oxide electrode, from theoretical grounds these may be expected to differ in their nature from those of the other two.

Where clear solutions of known salt concentration can be obtained without undue handling the application of indicators gives a fourth method. This is probably the only method of those mentioned which can be relied upon when dealing with soil extracts of *p*H value greater than 5·5. Although this method was invaluable to pioneering work such as that of Gillespie (15) the objections to the method as a means of obtaining the precise *p*H value of soils, particularly of alkaline (20) or saline soils, even with special and tedious technique, are too well known to need repetition. The colorimetric method was ruled out as unsuitable during the preliminary work and was not included in the subsequent standardisation, although in special cases it proved to be useful.

Table I.

Soil no.	Locality		Description		Soil horizon
			Texture	Type	
1089	Murray Bridge	S. Aus.	Heavy clay	Murray alluvium Reclaimed swamp	C
889	Trafalgar	Vic.	Fine sand	Podsol	A
196	Myponga	S. Aus.	Peaty	Swamp	A
1024	Renmark	S. Aus.	Clay	Murray alluvium	C
1030	Renmark	S. Aus.	Clay	Murray alluvium	C
1029	Renmark	S. Aus.	Clay	Murray alluvium	B 3
1028	Renmark	S. Aus.	Clay	Murray alluvium	B 3
203	Myponga	S. Aus.	Coarse sand	Podsolic	A
1033	Chaffey	S. Aus.	Fine sand	Murray alluvium	C
860	Glenorchy	Tas.	Fine sandy loam, gravelly	Faintly podsolic	A
146	Kuitpo forest	S. Aus.	Sandy loam	Podsolic on laterite	A
727	Everton	Vic.	Silty clay	Alluvial	A
9	Mt Pleasant	S. Aus.	Sandy loam	Faintly podsolic	A
906	Clifton	Q'ld	Loam (high clay content)	Red basaltic loam	A
U. 15	Glen Osmond	S. Aus.	Sandy loam	Brown earth	A
1037	Buln Gherin	Vic.	Sandy loam	Brown earth (on basalt)	A
5	Mt Pleasant	S. Aus.	Sandy loam	Faintly podsolic	A
K. Mt. G.	Mt Gambier	S. Aus.	Sandy silt loam	Volcanic ash, immature	A
1008	Bathurst	N.S.W.	Clay loam	Brown earth (basaltic)	A
854	Devonport	Tas.	Loam	Brown earth (basaltic)	B
459	County Hamley	S. Aus.	Sand	Mallee	A
856	Mowbray Swamp	Tas.	Peat	Fen	A
875	Werribee	Vic.	Sandy loam	Brown earth	A
161	Kuitpo forest	S. Aus.	Sand	Faintly podsolic	A
1017	Belalie North	S. Aus.	Sandy loam	Brown earth	A
897	Big Burleigh Hds.	Q'ld	Loam	Red basaltic loam	A
436	Morphett Vale	S. Aus.	Sandy loam	Brown earth	A
928	Bundaberg	Q'ld	Loam (high clay)	Red basaltic loam	A
927	Bundaberg	Q'ld	Loam (high clay)	Red basaltic loam	B
U. 113	Glen Osmond	S. Aus.	Clay	Brown earth	B 1
599	Naracoorte	S. Aus.	Clay	Brown earth (crab-holey)	A
1026	Renmark	S. Aus.	Light clay	Grey desert steppe soil	B 3
216	Koonamore	S. Aus.	Sandy loam	Mallee	A
600	Naracoorte	S. Aus.	Clay	Brown earth (crab-holey)	A
1044	Meteor Downs	Q'ld	Clay	Black earth (basaltic)	A
T. 1	Tresco	Vic.	Sandy loam	Mallee	A
348	Yurgo	S. Aus.	Sand	Mallee	A
915	Clifton	Q'ld	Clay	Black earth	A
587	Renmark	S. Aus.	Sandy loam	Grey desert steppe soil	A
T. 8	Tresco	Vic.	Sandy loam	Mallee	A
238	Pinnaroo	S. Aus.	Coarse sandy clay	Mallee	A
232	Pinnaroo	S. Aus.	Sandy clay	Mallee	A
G. 43	Griffith	N.S.W.	Loam	Mallee	A
T. 3	Tresco	Vic.	Sandy loam	Mallee	B
566	Renmark	S. Aus.	Fine sandy loam	Grey desert steppe soil	A
233	Pinnaroo	S. Aus.	Sandy clay	Mallee	B
G. 6	Griffith	N.S.W.	Clay loam	Mallee	B 2
630	Coomealla	N.S.W.	Sandy	Mallee	B 2
1065	Woorinen, Swan Hill	Vic.	Sandy clay	Mallee	B 2
G. 102	Griffith	N.S.W.	Loam	Mallee	B 2
598	Renmark	S. Aus.	Hardpan	Grey desert steppe soil	A 3
797	Ral Ral, Chaffey	S. Aus.	Sandy clay	Grey desert steppe soil	B 2
T. 14	Tresco	Vic.	Sandy clay	Mallee	C
T. 7	Tresco	Vic.	Sandy clay	Mallee	C
588	Renmark	S. Aus.	Sandy clay	Grey desert steppe soil	B 2

DESCRIPTION OF SOILS.

The soil material used was taken in most cases from the Waite Institute collection of air-dried samples which had been passed through a 2 mm. sieve. The soils used are described in Table I opposite the Waite

Institute number. The types are those tentatively proposed by Prescott[1]. Of these, the Mallee type appears to be characteristically Australian; these soils are usually alkaline and frequently saline in character—they are characterised by a relatively high proportion of Mg amongst the replaceable bases.

<div align="center">EXPERIMENTAL.</div>

<div align="center">MATERIALS.</div>

Chloroplatinic acid prepared from pure sheet platinum was used as a plating bath.

Hydrogen was drawn from a cylinder of commercial gas without further purification (other than passing it through water), because previous experience has shown that results obtained by such hydrogen differ from those obtained with hydrogen purified in the usual way[13] by less than 0·1 mv.

Mercury for the calomel electrodes was re-distilled after being purified chemically.

Calomel was prepared electrolytically[28].

The *potassium chloride* was A.R. quality re-crystallised from conductivity water.

Quinhydrone was prepared according to the method described by Biilmann and Lund[9]. Quinhydrone, prepared by direct combination of quinone and hydroquinone after Valeur[37], was found to give practically identical values with that prepared by Biilmann's method, and was therefore only used as a check on the freshness of the stock.

Antimony and Sb_2O_3 were the purest obtainable from the British Drug Houses, Ltd.

<div align="center">TECHNIQUE.</div>

<div align="center">(a) General.</div>

It is becoming increasingly evident that p*H* values have little value for comparative purposes outside the one investigation, unless the conditions under which they were determined are specified. On this account the technique employed is described as fully as space will permit, and this technique will be used as a basis for future work in these laboratories. Saturated KCl was used throughout for salt bridges, and was assumed to eliminate diffusion potentials.

Soil/water ratio. At the time the work was begun a soil/water ratio

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[1] Privately communicated. See also "A tentative soil map of Australia," *J. Coun. Sci. and Ind. Res., Comm. Aust.* (1930), **3,** 123.

of 1:1 was customary for routine determinations with the quinhydrone electrode. In many cases it is not possible to use such a concentrated suspension with the hydrogen electrode, and occasionally the paste is too stiff to be used comfortably with the quinhydrone electrode. As nearly all published work on the hydrogen electrode referred to a soil/water ratio of 1:5, this was adopted, and, unless otherwise stated, all values refer to this ratio. The difference in pH value between 1:1 and 1:5 suspensions of the soils tested varies from 0·1 to 0·5 unit. In the majority of soils tested the difference is approximately 0·3 unit, the higher value being obtained with the more dilute suspensions. In acid soils this higher value for a 1:5 suspension is readily understood as an undoubted dilution effect. In alkaline soils the increase in hydroxyl-ion concentration on dilution is probably due to an increase in the degree of hydrolysis with dilution, and in saline soils the salt effect is also reduced. The suspensions were made up with aerated distilled water of specific conductivity 2 to 3 × 10⁻⁶ mho. The pH value of this water was about 6, and it may be of interest to note that any great divergence of equilibrium water from this value must be due to impurities (other than CO_2) in the water[3]. After being shaken mechanically for 2 hours the suspensions were allowed to stand overnight in the constant temperature room in which the determinations were made. About 500 ml. of the suspensions were prepared and portions used for all determinations with the hydrogen and quinhydrone electrodes, and in most cases for the antimony oxide electrode as well. The results of the comparison are set out in Table II and Figs. 3 and 4.

Time of contact between soil and water. The pH value and conductivity of many colloidal solutions, notably hydrosols of the metals and their oxides, mastic and the like, change with time, and approach a dynamic equilibrium only after long standing. The soil-water system behaves similarly, although a true equilibrium may never be attained, and is complicated by the slow solution of mineral fragments and possible changes due to biological activity when the period is extended. Nevertheless, it is well known that the change which takes place between, say, 2 hours' and 24 hours' contact is very small, and it is generally assumed that values obtained after these intervals represent "equilibrium" values. For European soils a contact of 1 minute is usually regarded as long enough for the attainment of the equilibrium state, although a longer time is sometimes necessary[18]. Among the suspensions examined some do not alter appreciably after a contact of 1 minute. Some become more and others less acid on standing for 24 hours, alkaline

soils tending to become more acid. Except in extreme cases of sandy or saline soils this difference between the 1-minute and 24-hour value does not exceed 0·2 pH unit, but it is sufficiently large to make the 1-minute reading impracticable for Australian soils as a whole. Many of the drifts in E.M.F. noticed during the preliminary work with the quinhydrone electrode, in which readings were taken 1 minute after mixing soil and water, were traced to the fact that, while the reading was being taken, the pH value of the suspension was actually changing, equilibrium between soil and water not having been established. In general there is very little difference between values obtained after 10 minutes' and 24 hours' contact, and it is desirable that at least 10 minutes be allowed for the soil-water mixture to approach equilibrium. Any drifts due to lack of attainment of equilibrium are eliminated by adopting this procedure.

Effect of temperature on the pH *value of soils.* As the determinations were carried out in a room whose temperature varied only 0·2° approximately during a working day and, as portions of the same suspension were used for all methods, the temperature factor does not enter into the main question. However, as several measurements had to be repeated at a considerably later date, it became necessary to test the effect of changes of temperature on the pH value of the suspensions. Although there appears to be a general tendency for the pH value to decrease with rise of temperature, this decrease was small and often not significant over a range of 10°, except in the case of alkali soils. The maximum difference was obtained with soil No. 588; the pH values as determined by the Crowther electrode at 14°, 21° and 27° were 10·07, 9·98 and 9·87 respectively, showing a decrease of 0·20 unit for a rise of 13°.

When the quinhydrone electrode is used in these alkaline soils the effect of temperature is manifested in a different way, giving rise to a real error due to the greater solubility of quinhydrone at the higher temperature, thus setting free more hydroquinone to partake in the acid base equilibrium. This latter effect has also been noticed at Merbein, in northern Victoria, where laboratory temperatures vary over a considerable range. In the author's experience this error is only apparent in those soils to which the quinhydrone electrode is not applicable on account of their alkalinity (see later), and, moreover, the original error due to the participation of hydroquinone in the acid base equilibria even at 12° is much greater. For practical purposes, where values are required correct to 0·1 unit only, any alterations in the pH value of a soil caused by variations of temperature, within reasonable bounds, may be neglected.

It seems scarcely necessary to add that the pH values are all expressed at the temperature at which the determinations were made.

About two-thirds of the values listed in Table II were determined at or within 2° of 20° and with a few exceptions the remainder were determined at or within half a degree of 14°.

Standard electrodes. A saturated calomel electrode was used as a working standard and was periodically checked against a normal calomel half-cell and a Veibel(38) standard quinhydrone electrode. The electrode systems were periodically checked with standard buffer solutions.

(b) The hydrogen electrode.

The Crowther form of shaking electrode was used and proved to be highly satisfactory. A simple form of shaker based on a worm drive was employed, and is illustrated in Fig. 6. The technique employed was essentially that recommended by Crowther(12), except that the electrodes were plated with platinum and the E.M.F. measured while the vessel was at rest, the first reading being taken after the passage of the third lot of hydrogen. Electrodes were freshly plated at the beginning of each day. In most cases duplicates agreed to within 0·02 of a unit, and in the isolated cases where the difference exceeded 0·05 unit the determination was repeated. Only in the case of soil U. 113 was a serious difference obtained, the values for four successive determinations at one time and two at a later date being 6·65, 7·29, 6·65, 7·10 and 7·33, 7·30. It was noticed in one of the later determinations that the value obtained, if the second reading was taken after rocking (without passing a fourth lot of hydrogen), was lower by about 0·2 unit than in the other test, where the usual procedure of passing hydrogen before reading was observed. On passing hydrogen in the former case for a short while, the E.M.F. rose to within 2 mv. of the value obtained for the other test and remained steady so long as this procedure was adhered to. The quinhydrone and antimony oxide electrodes gave values of 7·22 and 7·33. The low values obtained in some instances may be due to the different catalytic activity of the coats of platinum black towards a trace of some depolarising agent present in the suspension, although the plating was carried out under apparently uniform conditions. Unfortunately, knowledge of surface actions is too limited to enable such cases to be predicted, but the example quoted goes to show that it would be unwise to rely absolutely even on the hydrogen electrode.

A form of *bubbling hydrogen electrode* as described by the author(4) elsewhere, which gives excellent results with other types of solutions

and attains equilibrium in a few minutes in buffer solutions, was tried out on soil suspensions, but in all cases low values (as compared with the Crowther electrode) were obtained. In weakly buffered suspensions even the reproducibility is very poor. In principle this vessel is similar to that described by Bray (6) and by Heintze and Crowther (17) for use with soil suspensions. Objections to its use for soils are that the KCl, which diffuses from the agar tube dipping directly into the suspension, will be uniformly dispersed by the stirring action of the hydrogen stream, and that the continuous bubbling of hydrogen may affect the carbonate equilibrium. It was to avoid these that the Crowther electrode was designed, and this electrode appears to be the soundest yet devised for soil work. Tests show that the lower values obtained by the bubbling electrode are largely due to the action of KCl, although the agar salt bridge is placed into position only just prior to taking a reading. Although this form of vessel may be used without any great error in certain types of soil, many Australian soils appear to be too susceptible to traces of KCl for its use. In the alkaline soils, Nos. 588, 797 and 1065, the values obtained were steady and reproducible, and were lower than the values obtained by the Crowther electrode (on portions of the same suspension) by 0·17, 0·22 and 0·24 unit respectively. Excellent results were obtained with this vessel in suspensions in N KCl where the carbonate equilibrium was not important, and the effect of KCl diffusion was negligible. As is to be expected, this electrode does not always give reliable results in weakly buffered KCl extracts of pH value about 6. For this reason the values as determined colorimetrically are given in Table IV for two of the supernatant liquids.

(c) *The quinhydrone electrode.*

The type of apparatus recommended by Biilmann and Jensen (8) was used. The "general instructions" issued to the committee appointed by the International Society of Soil Science to test out the quinhydrone electrode appeared in *Soil Research* (1) after the present work was completed, but the technique employed by the author was similar in all essentials to that recommended, except in the preparation of the suspension and the soil/water ratio. About 0·1 to 0·15 gm. quinhydrone was added to 10 ml. of the "equilibrium" suspension in a test tube, the whole being shaken by hand for 10 seconds. The test tube was then placed so that the electrode only was in contact with the suspension for 1 minute, after which the electrode was washed and dried lightly with filter paper, the suspension placed into position and the E.M.F. read

immediately (20 seconds) and at intervals of 1 minute. In practically all cases equilibrium was attained in less than 1 minute after the instantaneous reading, and this value was recorded. Those cases in which a potential drift occurred were dealt with individually.

Drifting potentials. The above procedure was adopted as a result of preliminary work in which the factors militating against steady and reproducible potentials were investigated. When the measurements were carried out 1 minute after mixing soil and water, potential drifts occurred in many instances, tending sometimes towards a lower and sometimes towards a higher pH value. They were found to be due chiefly to the following causes:

(a) lack of attainment of equilibrium between soil and medium;

(b) diffusion of KCl from the agar bridge into the neighbourhood of the electrode;

(c) an "adaptation lag."

The first of these has been dealt with earlier in the paper (pp. 341 and 342). Drifts due to (b) and (c) were investigated on equilibrium suspensions. No matter what precautions are taken in the preparation of the agar tube and the adjustment of the levels in the test tube and connecting vessel, diffusion of KCl must take place at the interface of a saturated solution of KCl and the soil suspension of relatively low salt concentration, and it is purely a question of the rate of diffusion, the distance between the interface and the electrode and the time elapsing between contact and reading the E.M.F., which will determine the magnitude of the salt effect at the electrode. Experience has shown that by placing the agar tube in the upper regions of the suspensions far removed from the electrode, as recommended by Bülmann and Jensen (8), this effect may be reduced to a minimum and may be neglected. It is also necessary to wash the tip of the agar tube and remove excess moisture with the aid of a strip of filter paper immediately before each determination. Another possible source of error due to KCl is the high acidity developed in some soils at the junction through exchange of K^+ and H^+ ions, leading to a diffusion or contact potential which may not be negligible. While determining the chloride present in a soil electrometrically by means of a Ag-AgCl electrode, the side tube of one quinhydrone standard half element, containing the HCl-KCl solution of pH value 2·03, was placed directly into a soil suspension in a beaker, and another similar cell was placed in contact with the same suspension through an agar-saturated KNO_3 salt bridge. The E.M.F. of the two cells against the Ag-AgCl electrode differed by as much as 10 mv., which must have been

chiefly due to a diffusion potential at the junction of the soil suspension and HCl-KCl buffer. This error also is minimised by the above procedure.

When a determination was made on a soil B immediately after the electrode had been used in a soil A of different pH value, the electrode being first carefully washed, there was a drift in potential towards the equilibrium value, beginning on the side nearer A. The equilibrium value reached after the drift was the same as that determined by means of a fresh electrode and agreed with the hydrogen electrode value. The greater the difference between the pH values of A and B, the greater the lag. This behaviour is quite general and similar to, but more serious than, that recorded by the author(5) for unbuffered solutions. For convenience this phenomenon will be referred to as an "adaptation lag." A thin invisible coating of soil colloidal material, which is not removed by the jet of water used to wash the electrodes, would account for such behaviour in soil suspensions, but does not account for similar behaviour in aqueous solutions of pure mineral acids. However, it is possible that, in both instances, an absorption layer of the first solution, which diffuses only slowly into the bulk of the second solution, is responsible for the lag in the attainment of equilibrium. The general behaviour lends qualitative support to this explanation. When consecutive soils differ in pH value by only 1 unit the effect is small, but for larger intervals its duration is a matter of minutes and its magnitude may approach $0.5\,pH$ unit. In general, the error due to this effect alone involved in taking an instantaneous reading instead of the equilibrium one would not exceed $0.2\,pH$ unit, but, by placing the electrode into the fresh suspension for a minute before making contact with the agar salt bridge, as previously outlined, this effect may be cut out entirely in most cases. It is only when the interval between two values is very large that more than 1 minute is required for the establishment of equilibrium. This effect is well illustrated in the higher value always obtained for the $M/20$ potassium hydrogen phthalate solution when checking the platinum electrode at the end of a day's work with alkaline soils. This phenomenon has also been observed by H. N. England (private communication) of the Griffith Research Station, N.S.W., and it would be interesting to know whether soils other than Australian behave similarly.

When drifts due to the causes enumerated above are reduced to a minimum, the potentials were steady and reproducible to within 0.01 unit for comparatively well buffered, and 0.05 for weakly buffered, soils. Under these conditions the 20-second and equilibrium readings agreed for all soils except poorly buffered sandy soils, which took from $\frac{1}{2}$ to

1 minute to reach a steady E.M.F. That the final reading in these cases is the correct one is seen from the E.M.F.-time drift curve (Fig. 1) of a soil typical of this class (No. 161), and from the fact that the equilibrium value approaches the hydrogen and antimony oxide electrode values. This may be regarded as a type of adaptation lag.
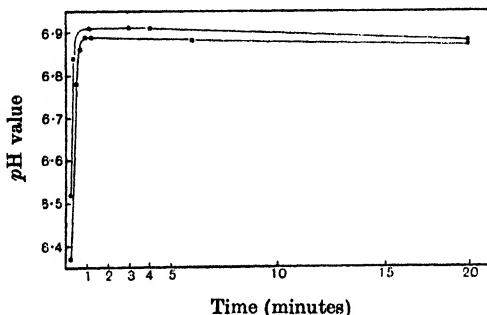
Fig. 1. Potential drift for a sandy soil (No. 161). Quinhydrone electrode.

(d) The antimony oxide electrode.

Metal-metal oxide electrodes have from time to time been recommended for acid-base titrations and control work, but so far only the antimony oxide electrode has been found to conform to the theoretical requirements of a simple oxide electrode.

This electrode was used as an indicator electrode for the titration of acids and bases by Uhl and Kestranck[36]. Kolthoff and Hartong[23] found that, by using two empirical equations connecting potential with pH value (one for solutions of pH value 1–5 and another for solutions of pH value greater than 9), fair correspondence with the hydrogen electrode values was obtained, but the electrode behaved erratically between pH values of 5 and 9. Addition of Sb_2O_3 and stirring of solutions were recommended. Franke and Willaman[14] employed the electrode for solutions met with in the paper-pulp industry and give an empirical equation to cover the range of pH values from 1–12. Snyder[34] applied a shaking form of the electrode to thirteen typical American soils, and, using the equation of Franke and Willaman, found the values so obtained to be in fair agreement with hydrogen electrode values. Lava and Hemedes[25] compared the electrode with the quinhydrone electrode in sugar-cane crusher juice and similar solutions, and in soil suspensions. Taking values obtained by the quinhydrone electrode as standard, they derived an empirical equation for the antimony oxide

electrode, which gave values in excellent agreement with the quinhydrone electrode for crusher juice, but for soils the agreement was not so satisfactory. No Sb_2O_3 was added to the solutions. The electrode has been satisfactorily applied to Indian soils by Harrison and Vridhackalam (16), and was used by them to determine the lime requirements of soils in the field.

Roberts and Fenwick (31) found that the potential of the antimony oxide electrode was a linear function of the pH value, with the theoretical slope from pH value 1 to 10, only provided certain requirements were satisfied, the chief ones being that any unstable solid phase (*e.g.* ortho-rhombic Sb_2O_3) must be absent, dissolved oxygen must be eliminated from the solution and the equilibrium must be approached from the alkaline side. The electrode took from 24 to 48 hours to reach equilibrium.

Obviously it is impossible to meet all these requirements when dealing with soil suspensions. It would appear from the work of the previous investigators and from the author's own experience that, under ordinary conditions, when the requirements laid down by Roberts and Fenwick do not prevail, side reactions take place and render the simple theoretical equation invalid. Until the nature and extent of these side reactions are definitely known, the relationship between pH value and potential of the electrode (when conditions are not ideal) can only be determined empirically, and this being so, empirical equations and calibration curves are only applicable to the particular set of conditions under which they were derived. A comparison of the equations derived by various investigators shows this very clearly. For purposes of comparison the constants have been re-calculated to apply to the chain

$$Sb \mid Sb_2O_3, \text{ test solution} \mid \text{Sat. KCl} \mid N \text{ KCl soln., HgCl} \mid Hg^+.$$

Investigator	Equation	Temperature (° C.)	Range of pH values over which equation applies	Type of solution
Kolthoff and Hartong	$E = 0.0415 + 0.0485\ pH$	14	1–5	Buffers
	$E = 0.009\ + 0.0536\ pH$	14	Above 9	,,
Franke and Willaman	$E = 0.050\ + 0.054\ pH$	25	1–12	Buffers and sulphiting liquors
Lava and Hemedes	$E = 0.052\ + 0.057\ pH$	26–29	—	Crusher juice and soils
Harrison and Vridhackalam	$E = 0.0649 + 0.0498\ pH$	30	4–9	Buffers and soils
The author	$E = 0.0549 + 0.0526\ pH$	27	Above 8	,,
	$E = 0.0532 + 0.0510\ pH$	20	,,	,,
	$E = 0.0615 + 0.0476\ pH$	14	,,	,,
	$E = 0.0512 + 0.0549\ pH$	27	5–7	,,
	$E = 0.0430 + 0.0513\ pH$	14	5–7	,,

Accordingly, in attempting to apply the method to soils, the conditions were simplified as much as possible. Although it is generally recognised that the electrode gives steadier values when the solution is agitated, it was felt that the shaking electrode gave no better values than could be obtained by the simpler method to be described, which has the advantage that it is better suited to routine work.

Preparation of electrodes and general procedure. Cylindrical sticks of antimony, some prepared by simply cutting the metal as supplied into convenient lengths (6 mm. × 40 mm.) and others by melting and recasting the metal into smaller sticks (4 × 30 mm.) in vertical moulds, were attached to stout pieces of copper wire by pushing the wire into position as the antimony solidified. These were than placed into pieces of glass tubing just wide enough to take the antimony rod, and the whole filled with paraffin wax. Although the two forms of rod behaved similarly, the smaller ones were found to be more convenient.

The procedure adopted to determine the potential difference set up at the electrode was similar to that employed with the quinhydrone electrode, the antimony rod taking the place of the platinum sheet, and antimony oxide replacing quinhydrone. When such an electrode was placed into a buffer solution, the E.M.F. remained fairly steady, there being in most cases a slight upward drift of about half a millivolt per minute (the antimony oxide electrode being the negative pole and a saturated calomel half-element being used as reference electrode). In many instances the drift was less pronounced, and in some cases, especially in acid solutions, there was a small drift in the opposite direction. On shaking the electrode by alternately raising and lowering it several times, the E.M.F. returned to the original value. Provided the readings were taken at approximately the same time after placing the electrode into the solution, the values were reproducible to within 1 mv., and seven out of nine different electrodes showed a maximum difference of 2 mv. from the mean over the whole range investigated (pH values 4–10). One of the two exception gave a constant difference from the mean of + 4 mv. and the other of − 3 mv. over the range of pH values 7–10. Both agreed with the mean below pH 7. Calibration curves at 14°, 20° and 27° were drawn by plotting the pH value against the E.M.F. of a number of antimony oxide electrodes (in conjunction with a standard cell) in a series of buffer solutions covering the pH range 4–10, made up according to the directions of W. M. Clark[10] and standardised with the hydrogen electrode. The form of the curve obtained is shown in Fig. 2. It resembles a titration curve somewhat with two straight lines

of different slope at its extreme ends. Although the equations given on p. 348 may be used to calculate the *p*H value over the range 8–10 and 5–7, the better method is to read off the *p*H value from the calibration curve, and this procedure was adopted. The large *temperature coefficient* cannot be disregarded, but the *p*H value for a reading at any temperature between 14° and 27° may be interpolated with fair accuracy from the curves by inspection.
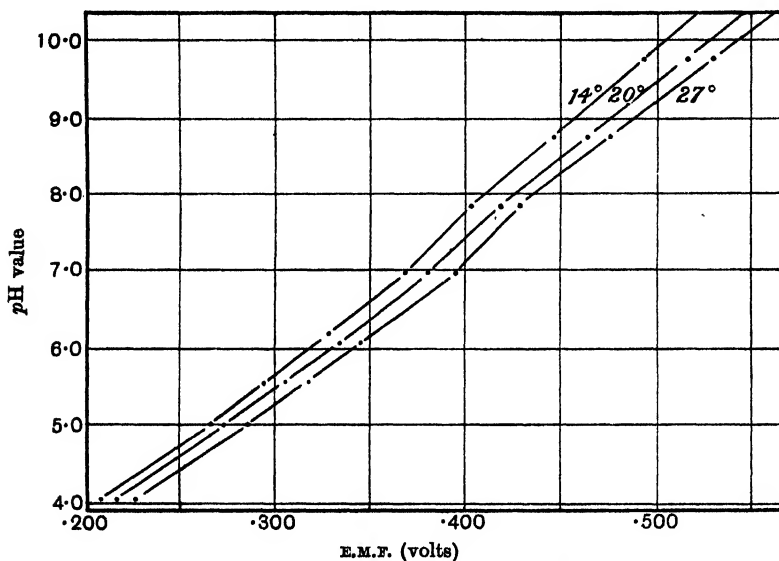
Fig. 2. Relationship between *p*H value and the corresponding E.M.F. of the antimony oxide electrode for 14°, 20° and 27°. A saturated calomel half-cell was used as reference electrode.

In order to test the application of the method to soils, a number of soil suspensions for which practically identical values had been obtained by the hydrogen and quinhydrone electrodes were prepared as before. The suspensions were arranged in order of their *p*H values, beginning with the most alkaline. A small quantity of Sb_2O_3 (enough to cover a disc 4 mm. in diameter) was added to approximately 15 ml. of the suspension in a wide test tube. After shaking, the suspension was stirred with the antimony oxide electrode to be used. After 1 minute the electrode was removed, washed with distilled water and any surplus moisture removed by lightly touching it with a fragment of filter paper. The electrode was then replaced and the E.M.F. read within half a minute and at intervals of half a minute, the equilibrium being approached

Table II. *Comparison of pH values obtained on a range of Australian soils by the hydrogen, quinhydrone and antimony oxide electrodes.*

pH value of aqueous suspensions, $\dfrac{\text{Soil}}{\text{Water}} = \dfrac{1}{5}$

Soil no.	Crowther hydrogen electrode	Quin-hydrone electrode	Antimony oxide electrode	Difference	
				Hydrogen-quinhydrone	Hydrogen antimony
1089	3·23	3·20	—	+ 0·03	—
889	4·29	4·25	4·38	+ 0·04	− 0·09
196	4·52	4·52	4·58	0·00	− 0·06
1024	4·76	4·73	—	+ 0·03	—
1030	4·85	4·85	—	0·00	—
1029	4·89	4·89	4·99	0·00	− 0·10
1028	5·11	5·30	—	− 0·19	—
203	5·41	5·33	—	+ 0·08	—
1033	5·47	5·42	—	+ 0·05	—
860	5·76	5·67	5·80	+ 0·09	− 0·04
146	5·77	5·63	—	+ 0·14	—
727	5·94	5·87	—	+ 0·07	—
9	6·07	5·94	—	+ 0·13	—
906	6·20	6·30	6·26	− 0·10	− 0·06
U. 15	6·36	6·93	6·39	− 0·57	− 0·03
1037	6·41	7·24	6·50	− 0·83	− 0·09
5	6·45	6·27	—	+ 0·18	—
K. Mt G.	6·60	6·48	—	+ 0·12	—
1008	6·66	7·76	6·63	− 1·10	+ 0·03
854	6·70	6·74	—	− 0·04	—
459	6·89	6·72	6·60	+ 0·17	+ 0·29
856	6·89	6·70	—	+ 0·19	—
875	6·97	6·81	—	+ 0·16	—
161	6·99	6·90	6·87	+ 0·09	—
1017	7·01	8·03	6·83	− 1·02	+ 0·18
897	7·01	6·91	6·72	+ 0·10	+ 0·29
436	7·05	7·07	6·86	− 0·02	+ 0·19
928	7·19	8·47	7·22	− 1·28	− 0·03
927	7·28	8·65	7·20	− 1·37	+ 0·08
U. 113	7·31	7·22	7·33	+ 0·09	− 0·02
599	7·36	7·56	7·27	− 0·20	+ 0·09
1026	7·96	8·39	8·24	− 0·43	− 0·28
216	8·02	8·03	7·90	− 0·01	+ 0·12
600	8·41	8·29	8·62	+ 0·12	− 0·21
1044	8·46	9·04	8·20	− 0·58	+ 0·26
T. 1	8·49	8·84	8·30	− 0·35	+ 0·19
348	8·68	8·28	8·60	+ 0·40	+ 0·08
915	8·80	8·89	8·90	− 0·09	− 0·10
587	8·83	8·68	—	+ 0·15	—
T. 8	8·88	8·87	8·67	+ 0·01	+ 0·21
238	8·94	8·86	8·90	+ 0·08	+ 0·04
232	8·94	8·51	9·03	+ 0·43	− 0·09
G. 43	9·11	9·02	8·95	+ 0·09	+ 0·16
T. 3	9·14	8·93	9·00	+ 0·21	+ 0·14
566	9·39	8·75	9·43	+ 0·64	− 0·04
233	9·41	8·76	9·53	+ 0·65	− 0·12
G. 6	9·43	8·99	9·24	+ 0·44	+ 0·19
630	9·62	8·96	9·60	+ 0·64	+ 0·02
1065	9·70	8·92	9·78	+ 0·78	− 0·08
G. 102	9·71	9·09	9·71	+ 0·62	0·00
598	9·79	8·88	9·78	+ 0·91	+ 0·01
797	9·92	9·04	10·00	+ 0·88	− 0·08
T. 14	9·95	9·20	9·96	+ 0·75	− 0·01
T. 7	10·00	9·28	10·04	+ 0·72	− 0·04
588	10·07	9·28	10·16	+ 0·79	− 0·09

from the alkaline side. If the drift after the first reading did not amount to more than 1 mv. per minute, the first reading was recorded. If the drift was more rapid (which happened only occasionally) the test was repeated.

When an antimony oxide electrode had been used in a solution of one pH value, and was then immediately washed and used in a solution of widely different pH value, there was a lag in the attainment of the "equilibrium" potential, analogous to the "adaptation lag" of the quinhydrone electrode. It is for this reason that the soil is first stirred with the electrode to be used in the determination. The pH value corresponding with the E.M.F. determined as described was read from the appropriate calibration curve. The agreement of the values so obtained with those of the hydrogen electrode was so good that the electrode was applied to determine the pH values of those soils for which the hydrogen and quinhydrone electrodes gave different values, and a number of other soils were added to the list and the pH value determined by the three methods.

In weakly buffered solutions, such as the local reservoir water, it has been found essential to add Sb_2O_3 to get a steady value at all. In the absence of the oxide the E.M.F. drifts rapidly, and even a forced reading gives results which are too high. For this reason the addition of Sb_2O_3 to sandy soils becomes essential, and it is safer to add it to all soils. The amount of salt normally present in soils, even in alkaline soils, does not appreciably affect the application of this electrode, but addition of sufficient KCl to buffer solutions causes the E.M.F. to drift more rapidly to higher apparent pH values. In suspensions of soil in N KCl solution the error due to this effect amounts to 0·3 pH unit, the antimony oxide electrode value being too high. This effect is probably due to slow formation of SbOCl. Where quinhydrone electrodes are used the same agar bridge cannot be used for determinations with the antimony oxide electrode, and all traces of quinhydrone must be excluded from the system, or low values will result. Stirring the solutions with hydrogen gas has been recommended, but where this was tried potentials drifted so rapidly that it was not possible to obtain a value at all. The hydrogen was evidently not "inert."

DISCUSSION OF RESULTS.

To show the correlation between the pH values obtained by the three methods, these are plotted against each other in pairs as dot diagrams, together with the theoretical line in Figs. 3 and 4. The antimony oxide

and hydrogen electrodes are seen to be in fair agreement over the whole range of soils investigated. Although the agreement is not always as good as could be desired, it is close enough to be of value in deciding between the hydrogen and quinhydrone electrode values where these differ, and it is also close enough to justify the use of the antimony oxide electrode for work in connection with soil surveys, where the
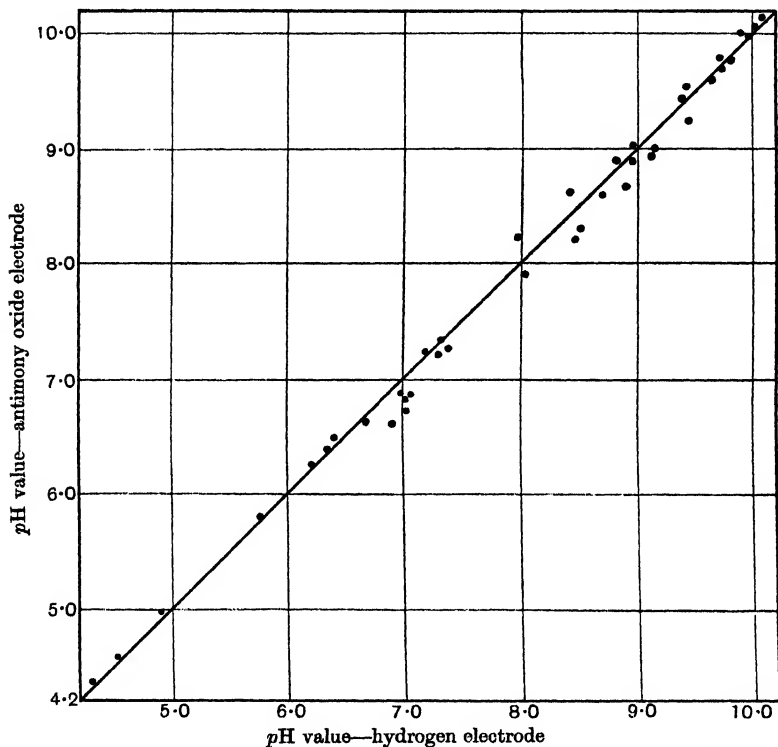
Fig. 3. Correlation between the pH values determined by the hydrogen and antimony oxide electrodes.

sampling error is probably much larger. For general physico-chemical work a method liable to give results in error to the extent of 0.2 pH unit would be useless for most purposes, but from general considerations such an error would not be regarded as serious for routine work with soils, although a larger error than 0.2 unit is undesirable even for such work. In working with the antimony oxide electrode, although three replicates are usually sufficient to fix the pH value to ± 0.05 unit, these may sometimes show a maximum difference amongst themselves as

great as 0·5 unit, and the measurement must be repeated a number of times to make sure that the values obtained are reproducible. By using an electrode of electrolytically deposited antimony on platinum and carefully purified Sb_2O_3, as used by Roberts and Fenwick, it may be
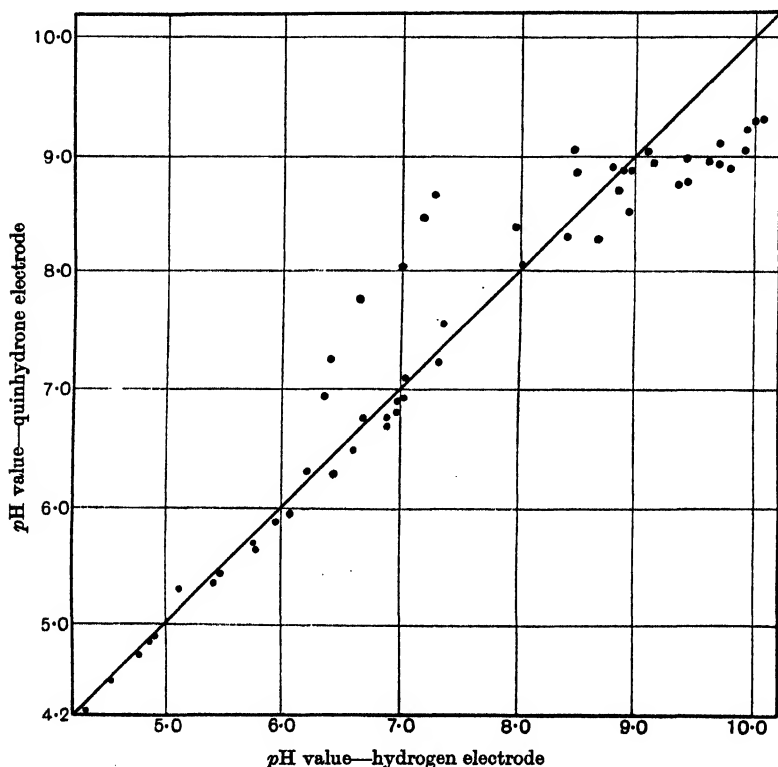
Fig. 4. Correlation between the pH values determined by the hydrogen and quinhydrone electrodes. Of the two types of deviation, the one for which the quinhydrone electrode values are higher than those of the hydrogen electrode is due to a reaction between MnO_2 and hydroquinone, and the other is due to hydroquinone taking part in the acid-base equilibria in alkaline suspensions.

possible to get a more dependable electrode, but, in view of the results obtained by the technique outlined, this did not seem to be an advantage.

From Table II and Fig. 4 it is seen that, over the range of pH values 3·2 to 5·5, there is good correlation between the hydrogen and quin-hydrone electrode values. With the exception of the half-dozen soils which lie well above the theoretical curve, there is fair correlation right up to pH value 8·5 or thereabouts. Above this value the two methods

give widely different results, and the higher the pH value the greater the divergence. The quino-quinhydrone and hydro-quinhydrone electrodes behave similarly in these alkaline soils, the divergence being even greater in the case of the latter. Similar differences between hydrogen and quinhydrone electrode values have been noted on South African alkaline soils [35]. This divergence with alkaline soils is to be expected because of the acid nature of hydroquinone, one of the fission products of quinhydrone, and Biilmann, La Mer and Parsons [24] and others [2] have drawn attention to this limitation. However, as it is often assumed that the quinhydrone electrode can be applied to such solutions and also to poorly buffered systems such as soil extracts, it seems desirable to stress this limitation of the method. In well-buffered solutions the electrode has been successfully used up to a pH value of 8·5 by many investigators. Rabinowitsch and Kargin [30] found that for poorly buffered solutions it gave lower values than the hydrogen electrode in solutions of pH value greater than 6. The author [5] has shown that the quinhydrone electrode may be used for totally unbuffered solutions of pH value 1 to 5, but that it gives erroneous results in such solutions with a pH value greater than 5. Where the accuracy required is no greater than $\pm 0·1$ unit, this limit may be extended to 5·5, when the proper precautions are taken. Further, from the known solubility and degree of dissociation of quinhydrone and K_1 for hydroquinone it can be calculated [22] that a saturated solution of quinhydrone in water has a pH value of 5·8. It is evident that the upper limit at which the acid nature of the hydroquinone will exert a disturbing influence will be set by the buffer capacity of the test solution. A large proportion of soil extracts may be classed as practically unbuffered solutions. Suspensions of Australian soils, while being much more highly buffered than their extracts, cannot on the whole be regarded as good buffers.

It follows that, for highly alkaline soils, the pH value indicated by the quinhydrone electrode may be expected to vary with the amount of quinhydrone added. This is actually the case, and a typical curve is shown in Fig. 5. In this case quinhydrone prepared by Valeur's method was used in order to rule out any possible action due to impurity often ascribed to the product as prepared by Biilmann's method. The pH values as determined by the hydrogen and antimony oxide electrodes were 10·07 and 10·16 respectively. The flattening of the curve is easily understood from general principles, apart from the nature of the ordinate unit. The change brought about in the quinone/hydroquinone ratio will also tend to cause a lower (apparent) pH value, but this effect is not

likely to be large. The pH value of normal soils was practically independent of the amount of quinhydrone added. As an example of the effect of hydroquinone on well-buffered solutions, a borate buffer (Clark(10)) gave with the hydrogen electrode a pH value of 10, and with the quinhydrone electrode 9·6. Of this difference 0·25 is accounted for by taking into consideration the effect of the ionisation of hydroquinone on the potential, by including the whole of the last term in the equation
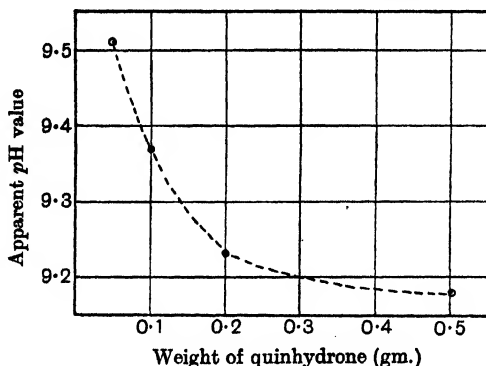
Fig. 5. Effect of varying the amount of quinhydrone on the apparent pH value of soil No. 588. The value obtained with the hydrogen electrode was 10·07.

connecting the potential of the quinhydrone electrode with the hydrogen-ion concentration, viz.

$$E_h = E^\circ{}_{QH} - \frac{RT}{2F} \ln \frac{[Hq]}{[Q]} + \frac{RT}{2F} \ln \{K_1 K_2 + K_1 [H^+] + [H^+]^2\}.$$

The symbols have their usual significance.

The remaining part of the discrepancy (which would be slightly greater but for a partial compensation due to the salt effect) must be due to a direct neutralisation of base by the hydroquinone, since the conditions of the determination precluded any effect due to auto-oxidation of hydroquinone. In this connection it is also interesting to note that a solution of pure NaOH of pH value 10 gave an apparent pH value of 8 with the quinhydrone electrode. Curves obtained by plotting apparent pH value against time for an alkaline soil of pH value 10 and an acid soil of pH value 4·9 show that, in the former case, effects due to auto-oxidation of hydroquinone are negligibly small during the time necessary for a determination, and in the latter case not detectable. It would appear that the hydroquinone set free by dissociation of quinhydrone takes part in the acid-base equilibrium, and actually alters the

pH value of the suspensions. The reason why some soils of pH value 9 do not show this effect is due partly to their being well buffered, but chiefly to a compensation of errors, the hydroquinone neutralising and producing free base simultaneously.

Amongst the original group of soils examined three only occurred in which the quinhydrone value was higher than that of the hydrogen electrode by more than 0·2 unit. The two outstanding ones were soils Nos. 928 and 1008, the differences being greater than 1 unit in both cases. Both soils are basaltic in origin, and therefore a larger number of basaltic soils were added at a later date to test the extent of the deviation in these soils. Immediately after measuring the pH value of soil No. 928 (the first soil of this class encountered), the electrodes of the hydrogen and quinhydrone systems were checked with a standard buffer solution. In all cases the expected value was obtained. Further, the suspension from the Crowther electrode vessel (after the hydrogen electrode determination was completed) was run off, and its pH value determined by means of the quinhydrone electrode. The values so obtained were the same as before. (This procedure was periodically adopted as a further means of checking.) As the antimony oxide electrode gave a value in close agreement with the hydrogen electrode, it is unlikely that a surface action causing a depolarisation of the hydrogen electrode was the cause of the discrepancy. Washing the soil with water did not remove the discrepancy. The remote possibility of the difference being due to a *reducing* agent capable of upsetting the hydroquinone/quinone ratio was ruled out because the quino-quinhydrone electrode gave practically identical values with the quinhydrone electrode on both washed and unwashed soils.

Whilst this work was in progress McGeorge[26] pointed out that some Hawaiian soils, known to contain a high percentage of MnO_2, gave higher pH values when determined by the quinhydrone electrode than when determined by the hydrogen electrode. More recently Heintze and Crowther[17] have drawn attention to a discrepancy between the hydrogen and quinhydrone electrode values, which was usually associated with a relatively high percentage of Mn both in the form soluble in dilute H_2SO_4 alone and in the presence of ferrous ammonium sulphate. Their explanation, that hydroquinone reduces MnO_2 to the more soluble $Mn(OH)_2$, which then actually alters the pH value of the suspension, is the most probable one so far put forward. Karraker[21] has reported discrepancies of a similar nature with manganiferous Kentucky soils. It is also well known that MnO_2 will decompose H_2O_2 vigorously, the reaction being

exothermic, and this reaction has been proposed by W. O. Robinson[32] as a test for MnO_2 in soils. Accordingly this test was applied to a number of normal and abnormal soils, which were also examined for Mn soluble in dilute H_2SO_4 alone and in the presence of ferrous ammonium sulphate by the method of Brewer and Carr[7]. Table III shows the rough correlation between the Mn content, rate of decomposition of H_2O_2 and the
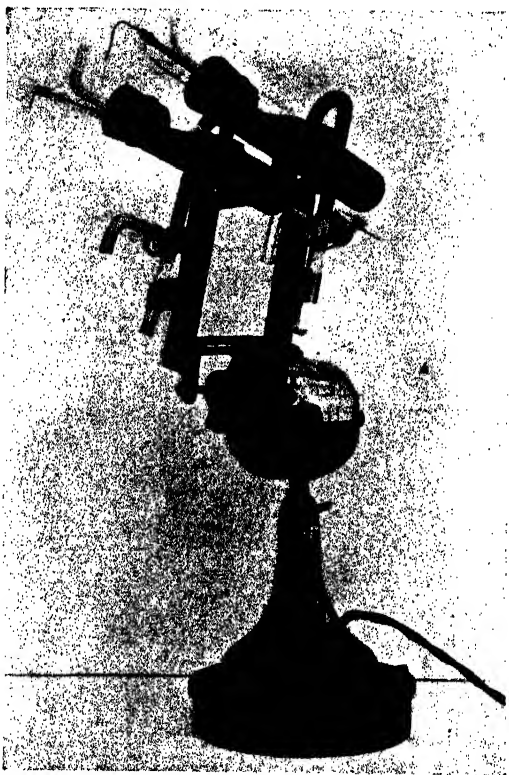
Fig. 6. Shaker for Crowther hydrogen electrodes.

deviation of the quinhydrone electrode value from that of the hydrogen electrode for a few typical cases of normal and abnormal soils. For the H_2O_2 test 10 ml. of neutralised reagent (100 vols. diluted with an equal quantity of water and adjusted to pH 7) were added to 2 gm. soil, and the rise in temperature in 1 minute determined approximately. The rise in temperature due to the heat of wetting did not exceed 1°. In most cases a rise of 4° or 5° in 1 minute was followed by a much greater rise

if the action was allowed to proceed for a longer period. It will be seen that, in general, serious differences between quinhydrone and hydrogen electrode values are associated with a relatively high percentage of manganese, although this is not always the case as instanced by the non-basaltic soil U. 115.

Table III. *Illustrating relationship between manganese content of soils and behaviour with quinhydrone electrode.*

% Mn (calc. to Mn₃O₄)		Reaction to H₂O₂		Error of quinhydrone electrode aqueous	Soil no.
Total	As "MnO₂"	Nature of O₂ evolution	Rise in temp. (° C.) in 1 min.	susp. ($\frac{1}{k}$)	
<0·01	<0·01	Weak to normal	0·4 to 1·8	+0·03 to −0·19	1024, 1028
0·01 to 0·025	Nil to 0·01	Normal	0·0 to 3	+0·12 to −0·02	903, 600, 436, U. 113
0·025 to 0·05	0·01 to 0·025	Weak to moderate	1·5 to 3	−0·10 to −0·43	1026, 906, 599
0·05 to 0·10	0·01 to 0·05	Weak to moderate	1·5 to 4	Nil to −0·57	U. 15, Q. 125, 907, 908
0·10 to 0·25	0·05 to 0·07	Moderate to brisk	4 to 8	+0·10 to −0·09	915, 918, 854, 897
0·25 to 0·50	0·14 to 0·17	Brisk	7 to 13⎱	> −1·0	⎰Q. 136, 1008
0·50 to 1·0	0·25 to 0·4	Violent	50 ⎰		⎱Q. 137, 929, 928, 927

From a practical point of view it would be an advantage to have some criterion whereby the manganiferous soils could be detected in the field. Although this may be done to some extent from their colour, this is not an infallible guide. Some of these soils exhibit a reddish chocolate colour. It is of interest to note that manganiferous soils described by Robinson[32] were similar in this respect. Some of the abnormal soils are red and others black, and although some of these latter soils change colour to a light brown after extraction with H_2O_2 in acid solution, this is not always so. In general, if a soil exhibits a chocolate-red colour it is likely to contain abnormal amounts of Mn compounds, and if red or black, the presence or absence of concretions (see p. 361) should help to decide. If it is possible to apply the H_2O_2 test, this is the most reliable. Another test for these soils is the fading out of the red colour and development of a blue colour when the Comber[11] test is applied.

In accordance with the practice of Heintze and Crowther[17], suspensions of representative soils were made in N KCl solution and allowed to come to equilibrium. (Soil/solution ratio was 1:5.) In this way a clear supernatant liquid could be drawn off from the soil with which it was in equilibrium, and in most cases the action of KCl brought the pH value into the region where the quinhydrone electrode could be used on the poorly buffered extracts. The results are set out in Table IV and are in accord with the experience of Heintze and Crowther. The hydrogen and quinhydrone electrodes gave comparable values for supernatant liquids and suspensions of normal soils. For abnormal soils they

agree on the supernatant liquids but differ on the suspensions, and for the latter the hydrogen electrode values agree with those obtained by both methods for the supernatant liquids. Colorimetric and antimony oxide electrode values agree with the hydrogen electrode values after allowing for a salt effect in both methods.

Table IV. *Comparison of the* pH *value of suspensions in* N *KCl solution by various methods.*

Soil no.	pH value of suspension			pH value of supernatant liquid	
	Bubbling hydrogen-electrode	Quin-hydrone electrode	Difference hydrogen-quinhydrone	Bubbling hydrogen-electrode	Quin-hydrone electrode
Q. 125	4·17	4·17	0·00	—	4·17
U. 113	5·16	5·26	− 0·10	5·3	5·25
U. 15	4·91	5·90	− 0·99	4·99	4·97
1008	4·99	6·52	− 1·53	4·96	5·27
Q. 136	4·82	6·47	− 1·65	—	4·78
				Colorimetrically	
Q. 137	5·78	7·74	− 1·96	5·9	
928	6·00	7·75	− 1·75	6·2	

In connection with aqueous suspensions it is of interest to note that, although the abnormal soils had reached steady and reproducible values under the conditions outlined on p. 344 for the quinhydrone electrode determinations, a marked drift occurred if the E.M.F. was read immediately after adding the quinhydrone. The drift was very rapid and could not be detected by the procedure adopted, as the quinhydrone was added 1 minute before taking the first reading. These soils were not encountered until the work was well under way and, therefore, the procedure outlined was continued for the sake of uniformity. The adaptation lag can be eliminated just as efficiently by placing the electrode into the soil suspension alone. The quinhydrone can then be added just before finally placing the test tube into position for the reading. If the procedure outlined on p. 344 is modified in this way, the 1-minute value may be taken as correct for all soils except where a decided drift has occurred during the first minute after adding the quinhydrone. Such a drift would be due either to the presence of MnO_2[1] or to the soil being very poorly buffered (cf. No. 161). In such cases the nature of the soil and the H_2O_2 or other test for abnormal soils will indicate whether the equilibrium values are reliable or not. Preferably a different method should be used as a check.

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[1] Wherever MnO_2 is used in the above connection it is understood that Mn_3O_4 and Mn_2O_3, if present, would behave similarly.

The form of combination of the Mn causing the disturbance.

Heintze and Crowther's work points to MnO_2 as the seat of the trouble. Crowther is of the opinion that a specially *active* form of MnO_2 is concerned in the reaction with hydroquinone. Brewer and Carr's method will give a measure of the MnO_2 in a mixture only when Mn_2O_3 and Mn_3O_4 are absent. Nevertheless, as any Mn_3O_4 and Mn_2O_3 may be expected to behave similarly with respect to reduction by hydroquinone (see *e.g.* Osugi and Kashihara (27)), the method gives a fair indication of the magnitude of the disturbing influence. It would appear that all the recorded effects can be accounted for by the presence of ordinary MnO_2 in the soil, although its state of division may affect the rate of reaction.

No exact correlation between amount of disturbing influence and the change in pH value brought about by it is to be expected. For the production of a given amount of base the effect on the pH value will vary with the initial pH value, and correlation must be in terms of hydrogen-ion concentration, if at all. A definite amount of MnO_2 will give rise, on reduction by hydroquinone, to an equivalent amount of $Mn(OH)_2$ and the effect on the hydrogen-ion concentration will depend in the first instance on the buffer capacity of the soil over the region concerned. Provided hydroquinone is present in excess of MnO_2 and the solubility product of $Mn(OH)_2$ is not exceeded before all the MnO_2 has been reduced, the change in hydrogen-ion concentration should be determinable if the amount of MnO_2 present, the buffer curve of the soil and the manganous-ion concentration are known. Where the amount of MnO_2 is high the limiting factor is the solubility of $Mn(OH)_2$, which decreases with increasing pH value, being inversely proportional to the square of the hydroxyl-ion concentration. There is evidence to show that this limit is reached in highly manganiferous soils before the MnO_2 has had full play. Buffer curves of a number of abnormal soils have been obtained, and comparison with the amount of $Mn(OH)_2$ which could be produced from the oxidised Mn present indicates that sufficient is present to bring about the observed changes.

Experiments planned to obtain definite evidence as to the forms of Mn occurring in the soil and causing the discrepancy are in progress, the following preliminary results being of interest. The coarser fractions of many basaltic soils show a marked pisolitic structure; small concretions, 2 mm. and less in diameter, are easily picked out from the mass, and on being crushed are found to consist in part of a black substance which exhibits the properties of MnO_2. A hand-picked sample of such

concretions was found to contain 10 per cent. Mn (total). Soil No. 928 was extracted with $0.2N$ H_2SO_4 alone and with $0.2N$ $H_2SO_4 + 5$ gm. ferrous ammonium sulphate. The amount of Mn extracted was determined, the residual soils being washed free of sulphate, dried, crushed and tested. For the sample extracted with H_2SO_4 + ferrous ammonium sulphate the reaction with H_2O_2 was very weak (weaker than with many normal soils), but the sample extracted with H_2SO_4 alone decomposed H_2O_2 briskly. Also the supernatant liquid of the former sample gave with the quinhydrone electrode the same value as the suspension, whereas for the latter sample the pH value as recorded by the quinhydrone electrode was higher for the suspension than for its supernatant liquid. Other portions of these samples were treated with NaOH to bring them to a pH value of about 6. The pH values of the aqueous suspensions were then determined by means of the quinhydrone and antimony oxide electrodes. The results (set out in Table V) taken in conjunction with the above facts show conclusively that it is the form of manganese which is insoluble in $0.2N$ H_2SO_4 but is capable of being reduced to a form soluble in dilute H_2SO_4, which is the cause of the trouble.

Table V. pH *value of aqueous suspensions of soil No.* 928 *after treatment with various extractants, washing and addition of NaOH solution.*

| | pH value | | |
Previously extracted with	Antimony oxide electrode	Quin- hydrone electrode	Difference: antimony- quinhydrone
$0.2 N$ H_2SO_4 + ferrous ammonium sulphate	5·59	5·46	+0·13
$0.2 N$ H_2SO_4 (hot)	5·84	6·93	−1·09
$0.2 N$ H_2SO_4 (cold) A[1]	5·67	6·30	−0·63
$0.2 N$ H_2SO_4 (cold) B	6·07	7·10	−1·03
Untreated soil	7·22	8·47	−1·25

[1] More NaOH was added to B than to A, hence the higher pH value recorded by the antimony oxide electrode.

Further preliminary work indicates that the reaction between MnO_2 and H_2O_2 varies with the pH value of the medium in which the reaction takes place. In alkaline solutions the decomposition of H_2O_2 is catalytic, the probable intermediate compound $Mn(OH)_2$ being re-oxidised to MnO_2. This reaction is well known, and may be represented by the following cycle:

$$MnO_2 + H_2O_2 \rightarrow Mn(OH)_2 + O_2,$$

$$Mn(OH)_2 + H_2O_2 \rightarrow MnO_2H_2O + H_2O.$$
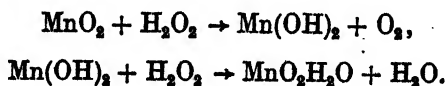
In acid media, however, the MnO_2 is speedily dissolved owing to reduction to manganous ion and solution of this form in the medium. This work has definitely established the stoichiometric nature of the reaction which takes place in accordance with the equation:

$$\overset{++++}{Mn}O_2 + H_2O_2 + H_2SO_4 \rightarrow \overset{++}{Mn}SO_4 + 2H_2O + O_2.$$
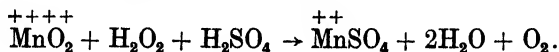
This reaction has been used by the author to determine the amount of reducible manganese in soils, and recently a paper by Schollenberger[33] has appeared where H_2O_2 is added to an acidified soil suspension to extract MnO_2. The above reaction may be made the basis of a quick method for the approximate determination of the amount of "MnO_2" in soils. The soil suspension is acidified with concentrated H_2SO_4 and a convenient amount of standardised H_2O_2 added. After a few minutes the soil is filtered and the excess H_2O_2 is determined by titrating aliquots of the filtrate with $KMnO_4$ solution. The method is only approximate because of a slight decomposition of H_2O_2 by constituents of the soil other than MnO_2, but where MnO_2 is present in excess of 0·1 per cent. the method has been found very useful.

Hydroquinone may also be used to bring the oxidised Mn into solution. The amounts of Mn brought into solution by extracting soil No. 928 with hot $0·2 N$ H_2SO_4 alone and in the presence of H_2O_2, hydroquinone and ferrous ammonium sulphate, were 0·44, 0·82, 0·83 and 0·83 per cent. (calculated to Mn_3O_4) respectively. The fact that the same amount of Mn is brought into solution by the three different reducing agents leaves no doubt that Mn in a definite state is being determined.

CONCLUSIONS.

No one method for determining the pH value of soils can be regarded as being universally applicable, but the Crowther modification of the Clark hydrogen electrode approaches this ideal very closely.

The quinhydrone electrode is inapplicable to soils of pH value greater than 8·6 (the precise limit for any soil type can only be fixed by experiment), and to certain soils containing Mn in a highly oxidised state, chiefly as MnO_2. The latter class of soil is usually easy to detect by the H_2O_2 reaction. For routine work it is necessary that the various methods be first checked on type samples. If the comparison is satisfactory it is recommended that the quinhydrone electrode be used throughout the series unless a pH value greater than 8·6 is recorded or the soil reaction with H_2O_2 indicates the presence of MnO_2, in which cases the antimony

oxide electrode should be used. The antimony oxide electrode is particularly useful in alkaline soils. The characteristic brown discoloration due to oxidation products derived from hydroquinone is apparent in the supernatant liquids of alkaline and manganiferous soils, and serves as an additional warning of the inapplicability of the quinhydrone electrode.

Although an ideal method which will combine reasonable accuracy with speed of operation and universal applicability has so far not been forthcoming, this ideal may be approached by a judicious choice of the various methods available and occasional checking of one against the other.

<div align="center">SUMMARY.</div>

1. The antimony oxide electrode has been applied in a simple form to measure the pH value of soils.

2. The pH values of representative Australian soils have been determined by means of the hydrogen, quinhydrone and antimony oxide electrodes and the results compared.

3. The limitations of the quinhydrone electrode have been investigated and their nature elucidated, special attention being paid to alkaline and manganiferous soils.

4. The nature of potential drifts has been investigated together with methods of minimising them.

5. The *adaptation lag* of the quinhydrone electrode has been found to reach more serious proportions in soils than in solutions of pure mineral acids, and the antimony oxide electrode was found to be subject to the same lag.

6. It has been definitely shown that Mn in an oxidised state is responsible for the non-applicability of the quinhydrone electrode to some Australian basaltic soils.

7. Data bearing on the nature of Mn oxides in the soil are presented and discussed.

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STUDIES IN SAMPLING TECHNIQUE: CEREAL EXPERIMENTS.

I. FIELD TECHNIQUE.

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(With One Text-figure.)

PRELIMINARY experiments carried out in the summer of 1928 had indicated that sampling methods might be used to obtain a satisfactorily precise estimate of the yield of small cereal plots[1]. It was thought desirable to test the method more thoroughly, and to compare the estimates with figures obtained by large-scale methods. Accordingly samples were taken from all the plots of the three Rothamsted cereal experiments of 1928, the plots later being harvested with a binder in the ordinary manner. Samples were also taken from the 16 plots of a small experiment on barley at Wellingore, Lincs., but no direct measurement of total yield was made for these plots. There were 210 plots in all, but of these 50 were sampled in four sections, corresponding with minor differences in manurial treatment; so that the total number of plots dealt with amounted to 360. The large-scale method, however, treated each of the 50 as a single plot, and thus provided only 210 yield figures.

THE METHOD.

The sampling method was Method (*a*) of the earlier paper on cereals[1]: 20–32 metre-lengths of drill were cut at randomly located points in each plot, with the restriction that half the number should be cut from each half of the plot; or, in the case of the 50 Rothamsted barley plots, one-quarter of the number from each of the four sections into which the plots were divided. Since the metre-lengths, apart from this restriction, were located independently, they represent the constituent "sampling-units" of the sample[2].

NUMBER OF SAMPLING-UNITS.

The earlier work had shown that 30 metre-lengths of drill from a plot 1/40th acre in area, might be expected to give a yield estimate with a standard error of about 5 per cent. This figure would vary comparatively

little with the size of the plot over a considerable range, unless the field were very heterogeneous; as the area increased, slightly larger samples would be required to give the same accuracy. It was therefore decided to take 30 metre-lengths from the 48 plots of the oats experiment, whose area was 1/40th acre; and 8 from each quarter, or 32 in all, from each of the 50 plots of the barley experiment, where the area was again 1/40th acre. The 96 plots of the wheat experiment were only of about 1/55th acre, and it was considered sufficient to take only 24 metre-lengths from each.

It will be noticed that only 8 metre-lengths were taken from each quarter of the barley plots, whereas to get a yield estimate with standard error as low as 5 per cent. a considerably larger number would have been necessary. The reason for this apparent change of standard is best seen from a consideration of the plan of the experiment. There were two 5×5 Latin squares, giving 50 plots of 1/40th acre. In one of the squares the treatments were:

1. No nitrogen
2. Sulphate of ammonia ⎫
3. Muriate of ammonia ⎬ To give 0·2 cwt. of N per acre.
4. Cyanamide
5. Nitrate of soda ⎭

In the second square urea replaced "no nitrogen," and the dressings were at the rate of 0·4 cwt. of N per acre.

The arrangement of the plots in Latin squares ensured that each treatment should occur on five different plots.

Now one quarter of each plot received no further treatment; a second quarter received muriate of potash at the rate of 0·5 cwt. K_2O per acre; a third superphosphate at the rate of 0·6 cwt. of P_2O_5 per acre; and a fourth both these treatments. Then the direct comparison of the effects of potash and phosphate is made between means of 100 quarter-plots; while interactions between these manures and the various nitrogenous treatments will be tested on means of 20 quarter-plots (except with "no nitrogen" and "urea," where there are only 10). These numbers are halved where the interactions between potash and phosphate are being studied, or where the two levels of nitrogenous manuring are treated separately.

Now since these large numbers of quarter-plots contribute to the various comparisons, individual estimates of yield from the quarter-plots need not be found with so high a degree of accuracy as from the whole

plots. It was consequently considered sufficient to take only 8 metre-lengths from each. The justification for this lowering of the standard of accuracy will be seen in the results.

<div align="center">STRUCTURE OF SAMPLING-UNITS.</div>

The sampling-unit, as has already been stated, was a metre-length of drill. The constituent half-metres were not, however, contiguous, but were separated by an interval of one metre. The measuring-rod was thus of the form shown in the figure. Horizontally projecting nails marked the ends of the half-metre-lengths which were to be cut.

$\frac{1}{2}$ metre	1 metre	$\frac{1}{2}$ metre
Cut	Left	Cut
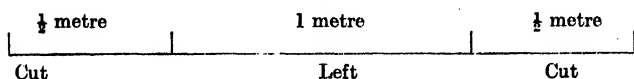
The advantage gained by this division of each sampling-unit into two separated units lies in the increased representativeness of the resulting sample, and can be measured directly by appropriate statistical methods, provided that the weights of produce from the individual units are recorded. This was not done in the present series of experiments, but had previously been done on a number of occasions, the results then obtained justifying the method. The statistical procedure is an analysis of variance, and consists in comparing the variation *between* whole-metre-lengths with that between their constituent half-metre-lengths: that is, with that *within* metre-lengths. If the former is the greater to an extent, which would not often occur merely by chance, it is concluded that more information about the crop would have been obtained had the half-metres been completely scattered, instead of being grouped in closely associated pairs. If on the other hand the two variations do not differ significantly, it is concluded that no information has been lost by the association.

The actual test experiments consisted in comparing the variation between and within metre-lengths; first, where constituent half-metres were immediately contiguous; and secondly, where an interval separated them. The measure of variation employed is R. A. Fisher's "Mean Square," and comparisons are effected by means of the "z" test [3].

As an example, the analysis is given for counts of shoot-number made on June 29th, 1928. 32 metre-lengths of drill were selected at random from a small plot of wheat, and separate counts were made of the shoots in each half-metre-length. There were thus 64 observations in all, and the 63 degrees of freedom were divided into 31 for the differences between

whole-metre lengths, and 32 for differences between the constituent half-metre-lengths of the same metre-length.

	Degrees of freedom	Sum of squares	Mean square	z
Between metre-lengths	31	1733·7	55·93	—
Within metre-lengths	32	725·5	22·67	0·4514
Total	63	2459·2	—	—

The 5 per cent. point of z is 0·2361, so that mean square "Between metre-lengths" is significantly greater than that "Within metre-lengths," and there is a loss of information as compared with that obtainable from the same number (64) of independently located half-metres.

On July 5, 1928, counts of ear-number were made on the same wheat, but using a dissected 4-ft.-length instead of a metre-length of drill as the observational unit (Fig. 1).

1 ft.	2 ft.	1 ft.	2 ft.	1 ft.	2 ft.	1 ft.
Count	Omit	Count	Omit	Count	Omit	Count

Fig. 1.

	Degrees of freedom	Sum of squares	Mean square	z
Between 4-ft.-lengths	16	361·37	22·59	—
Within 4-ft.-lengths (=between 1-ft.-lengths in the same 4-ft.-length)	96	1811·75	18·87	0·0900

Here the 5 per cent. point of z is 0·2763, and 1-ft.-lengths within the same 4-ft.-length do not resemble each other appreciably more than do 1-ft.-lengths from different 4-ft.-lengths. There is thus no loss of information when the increased labour of completely independent location is avoided.

A similar result was obtained when the sampling-unit consisted of two half-metres separated by a metre, and this pattern was accordingly adopted.

LOCATION OF SAMPLING-UNITS.

In determining the points at which sampling-units were to be cut, two numbers were chosen, one representing a drill-row, and the other a distance along the plot in paces. If there were n drill-rows in a plot, and 30 sampling-units were required from each plot, 30 numbers from 1 to $(n - 2)$ were chosen at random, by the use of Tippett's "Tables of Random Sampling Numbers"[5]. To each of these was added 1, so that there were 30 numbers ranging from 2 to $(n - 1)$, representing 30 independent selections of a row other than an edge-row (the number of the

row being counted from the edge-row for convenience). Now to each of the first 15 there was assigned at random a number from 1 to $(m-1)$ and to each of the second 15 a number from 0 to $(m-2)$, where m is the length of a half-plot, in paces. This ensured that no crop should be cut within 1 pace from the ends of the plot. The first 15 pairs of numbers were then allocated to one half-plot, and the remaining 15 to the other half-plot. The procedure in the field was then to start at the end of the plot and walk the required number of paces down a certain row, and to place the measuring-rod with its end just touching the toe of the forward foot. To minimise trampling of the crop, the pairs of numbers for each half-plot were arranged in ascending order of paces along the plot, so that there was steady progress in this direction, although it was still necessary to cross from one row to another. When the 15 metre-lengths had been taken from one half-plot, the counting of paces for the second set of 15 was started from the middle of the plot—*i.e.* at the boundary between the half-plots.

HARVESTING OF THE SAMPLING-UNITS.

The produce of each sampling-unit was cut about an inch above the ground with large scissors, and the two half-metre-lengths tied together into a single sheaflet. Before tying the heads were thrust into a perforated paper bag, and this was secured by means of the string of a label which indicated the plot and the serial number of the sampling-unit. It was found unnecessary to tie at any other point since the bag covered about a third of the length of the sheaflet. The sheaflets from a plot were tied into a single sheaf, which was suspended from the roof of a well-ventilated room.

The paper bags were 7-lb. sugar bags, as supplied to grocers, and were of fairly thick and strong yellow paper, glazed externally. Thirty-two holes, just small enough to prevent a cereal grain from passing, were punched in each bag, to ensure rapid drying of the heads.

A few samples were found to be mouldy when examined before threshing, but these were in all cases very weedy, and were probably cut when the corn was slightly damp. It is expected that with an increase in the number of perforations, and more care in cutting only when the crop is quite dry, there will be no trouble from this cause.

WEIGHING AND THRESHING.

The sheaflets were weighed before threshing, and the grain after threshing, the difference, corrected for the weight of the bag, string and label, being taken as straw. The balances were supplied by Messrs W. and T. Avery, of Birmingham, and were direct-reading machines with charts graduated from 0 to 100 gm. at intervals of 1 gm. An adjustable air-damping device made weighing a very rapid process, since the pointer was almost dead beat.

The electrically driven bench thresher and winnower is described in the next paper.

LABOUR.

Table I compares the labour expended per plot in harvesting the three Rothamsted experiments by large-scale and by sampling methods respectively. The large-scale methods consisted in cutting with a binder, stooking, carting to the threshing machine, threshing, and weighing the produce. Time spent in supervising these operations is also included in the estimates. It must be borne in mind, however, that the exceptionally dry summer of 1929 made it possible to thresh all experimental produce in the field, the labour of stacking being entirely saved and that of carting much reduced in consequence. It is estimated that the large-scale figures would be almost doubled in a normal season.

Table I. *Man-hours per plot.*

	Large-scale method	Sampling method
1. Oats experiment	1·67	2·25
2. Wheat experiment	1·69	1·95
3. Barley experiment	2·18	2·36

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STUDIES IN SAMPLING TECHNIQUE: CEREAL EXPERIMENTS.

II. A SMALL-SCALE THRESHING AND WINNOWING MACHINE.

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(With Plate II and Two Text-figures.)

On the adoption of a sampling method for the estimation of the yield of small experimental plots, the need arose for a threshing machine capable of threshing quickly and accurately the small sampling-units which comprise the sample from an individual plot. These sampling-units contain various numbers of ears from some three or four to twenty or more, and there is no commercial machine which deals adequately with such small quantities of grain. The number of sampling-units from a typical randomised block experiment may amount to 2500, and to thresh and winnow such numbers in two distinct operations would take no inconsiderable time. As a direct result of this, a machine was constructed in which these two operations were successively performed, without the need of handling the intermediate products. It is the object of this paper to describe this machine and the manner in which it was used for this purpose at Rothamsted last season. Wheat, oats and barley were treated, only minor alterations of the size of the screens, the speed of rotation and the strength of the blast for the separation of the chaff being necessary.

The machine depicted in Fig. 1, in its essentials like the ordinary commercial machine, consists of two main parts, the actual beating arrangement shown in the lower photograph, and a reciprocating screening attachment shown in vertical section in Fig. 2. The overall length of the completed thresher is some 3 ft., its breadth is about 10 in., and the height about 4 ft.

The ears, on the straw, are fed into the machine by the door at A, long straw and the fraction often styled "pulse" in large-scale operations are ejected at B, while the chaff is blown out at C. The dressed grain is collected in the small drawer at D. With suitable adjustments of the

speed of rotation of the drum, and of the size of the screens, and their rate of reciprocation, coupled with a favourable blast from the blower at E, the sample of dressed grain is practically free from extraneous matter. The most difficult cereal to thresh and winnow clean was found to be barley, some trouble in removing all the awns being experienced.

The actual separation of the grain from the ear is effected by the revolving toothed cylinder, rotating at fairly high speeds in a similarly toothed concave shown in the lower photograph (Pl. II)[1]. The distance
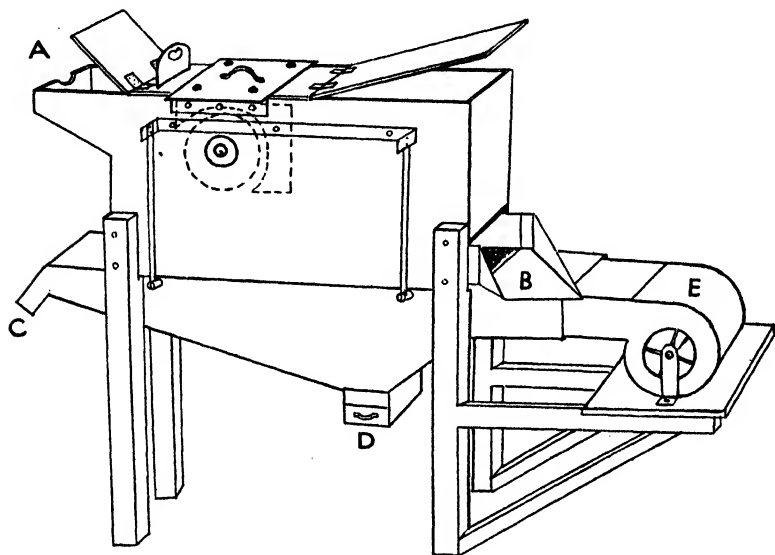
Fig. 1.

between the concave and the drum can be adjusted according to the type of corn which is to be threshed. The space between adjacent teeth is some $\frac{5}{8}$ in. while the teeth are approximately $1\frac{1}{4}$ in. long, and $\frac{1}{4}$ in. in diameter. The mixture of grain, straw, and chaff falls upon the riddle A (Fig. 2) of the screening device. The reciprocating motion for this attachment is obtained from a crank situated at the feeding end of the machine, and driven by a belt from the shaft of the drum at about two-sevenths the speed of the latter, the throw of the crank being some 3 in. The grain, chaff, weed seeds, etc., fall through the screen on to the solid part B,

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[1] Messrs Garvie of Aberdeen supply a "Bench Thresher" whose working part consists of a toothed drum revolving in a toothed concave much smaller than that used at Rothamsted. This was not found satisfactory.

the straw and empty ears are shaken off at *D*, the grain and chaff passing over the edge of this part to meet the air blast entering at *C*. The heavier grain is shaken through the riddle *G*, the lighter empty glumes, awns, etc. are ejected through the opening *E*. The grain is collected in the drawer *F*, which is fitted with a removable screen at the bottom. The mesh of this screen is of such dimensions as just to retain the size of grain it is desired to collect; small corn, weed seeds and other impurities are by this means eliminated.

The power for the thresher was supplied by a 1 H.P. electric motor running at a maximum speed of some 2000 R.P.M. Alteration of the size of the driving pulleys gave suitable speeds for the threshing of different cereals. The blower consisted of a four-bladed rotor driven by an electric
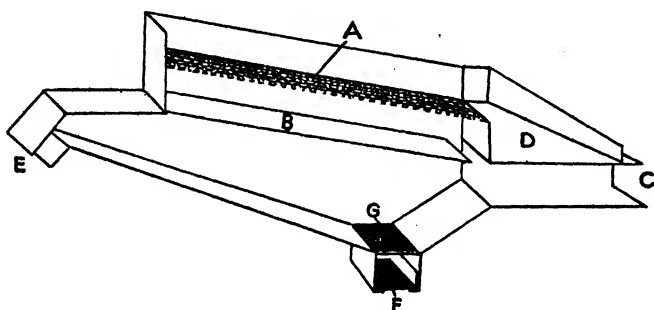
Fig. 2.

motor which was directly coupled to it, revolving at some 2000 R.P.M., giving a calculated output of 220 cu. ft. per minute. The strength of the blast was varied by altering the speed of the motor, a small resistance being used for this purpose.

To attain the maximum speed in dealing with the samples, four operators were needed, and the work was divided among them in the following manner. Having suitably arranged them in a definite order, the first worker weighed the samples and recorded their weights. He then removed the paper bags enveloping the heads of each individual sample, and laid the heads, together with any loose grains upon cardboard trays which he placed ready for the "feeder." The feeding of the machine required the almost undivided attention of another worker. The third was employed in starting and stopping the machine at the beginning and conclusion of each sample, and in removing the full drawers and inserting empty ones. The last worker weighed the dressed grain and recorded its weight. For all weighings Avery A 534 direct-

reading balances were used, their high degree of damping greatly facilitating the speed with which the samples were handled.
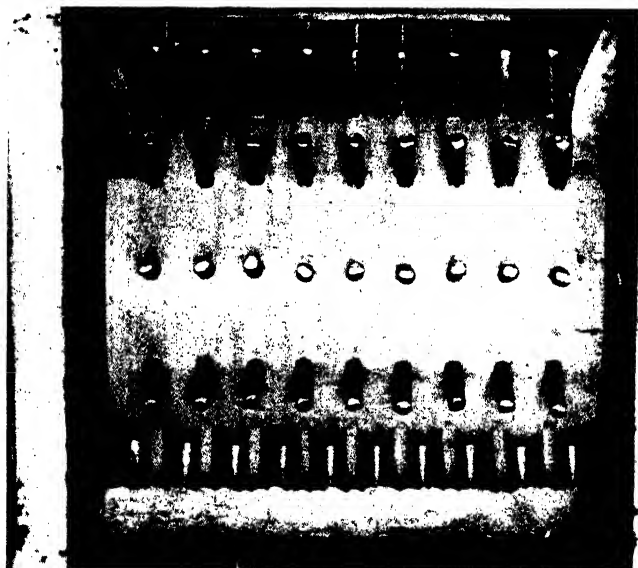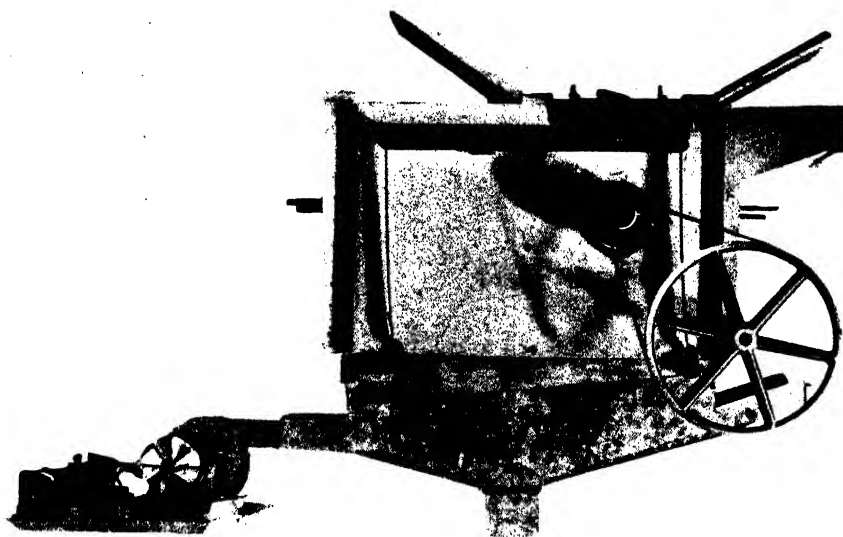
With such a disposition of labour it was possible to thresh and finish some 60 separate samples per hour (for one or two short periods a speed of 84 per hour was reached), an achievement which is in itself sufficient testimony to the value of this machine. As mentioned above, barley offered the greatest difficulty, but by the introduction of several minor improvements it is hoped that these difficulties have been overcome.

The upper photograph (Pl. II) shows the machine without driving belt and supports.

STUDIES IN SAMPLING TECHNIQUE: CEREAL EXPERIMENTS.

III. RESULTS AND DISCUSSION.

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(With One Text-figure.)

AFTER threshing there were three sets of figures, representing total weights of sheaflets, weights of grain, and weights of straw. These were subjected to an analysis which aimed firstly at obtaining a direct estimate of the sampling-error, and secondly at comparing the significant results of the experiment, treated as a manurial trial, with those obtained from the "total yields." The analysis was also designed to show whether it was really of value to divide the plot into two or more parts from each of which equal numbers of metre-lengths were cut.

The first step consisted in summing sheaflet yields (whether total, of grain or of straw) and squares of yields, for each half-plot and plot. The plot totals were then compounded in various ways to give sums of sheaflet yields and of squares of sheaflet yields for each block of plots and for each treatment. The grand totals of sheaflet yields and of sums of squares were also calculated.

From these quantities it is no difficult matter to find the total sum of squares of deviations of sheaflet yields from the mean sheaflet yield. This total can be divided into two parts, one representing variations *between* and one *within* half-plots. The former can be further divided into variations *between* different plots, and variations between half-plots *within* the same plot. The variation between plots is now divisible into fractions representing variation between blocks of plots; variation between manurial treatments; and lastly, variation due to uncontrolled causes, such as differences in soil fertility between plots in the same block; errors of area measurement, of weighing the manures, etc.; and errors due to sampling. This last fraction affords a basis for estimating the standard error of treatment comparisons.

The labour of calculation was much lessened by the use of a calculating machine.

1. ROTHAMSTED WINTER OATS EXPERIMENT.

In this experiment there were sixteen different manurial treatments, and each occurred three times, once in each of three compact "blocks" of plots, the position within the block being assigned at random. The arrangement was therefore an example of Fisher's "Randomised Blocks" method for field experimentation. The sixteen treatments were selected to give as much information as possible about the relative values of sulphate of ammonia and cyanamide, both as spring and autumn dressings. The unit dressing was in all cases equivalent to ¾ cwt. per acre of cyanamide with 19·0 per cent. N. One plot in each block received no dressing; four received single units; six received pairs of different units; four received three different units; and one received all four units. The table shows the manurial scheme.

Table I.

Treatment no.	Treatment				Treatment no.	Treatment			
1	—	—	—	—	9	—	Ss	Ca	—
2	Sa	—	—	—	10	—	Ss	—	Cs
3	—	Ss	—	—	11	—	—	Ca	Cs
4	—	—	Ca	—	12	Sa	Ss	Ca	—
5	—	—	—	Cs	13	Sa	Ss	—	Cs
6	Sa	Ss	—	—	14	Sa	—	Ca	Cs
7	Sa	—	Ca	—	15	—	Ss	Ca	Cs
8	Sa	—	—	Cs	16	Sa	Ss	Ca	Cs

Sa = Sulphate of ammonia applied in autumn.
Ca = Cyanamide applied in autumn.
Ss = Sulphate of ammonia applied in spring.
Cs = Cyanamide applied in spring.

By selection of the appropriate groups of plots it is possible to find the effect of each unit dressing separately, and their "interactions" when two, three or four are present. Every plot can be used for each of these comparisons, so that the arrangement is one of high efficiency.

As has already been stated, the plots, 48 in number, were each 1/40th acre in area, and 30 metre-lengths (15 from each half-plot) were cut from each.

Tables II and III give the complete analyses for grain and straw, both for yields estimated from samples and for "actual yields," obtained by the use of large-scale methods.

The entries in the column headed "Mean square" are obtained from those in the "Sum of squares" column by dividing by the appropriate number of degrees of freedom.

Dealing first with the analysis of "sampling yields," comparison of

Table II.

Sampling yields (gm. per sampling-unit).

Fraction	Degrees of freedom	Grain		Straw	
		Sum of squares	Mean square	Sum of squares	Mean square
Blocks	2	1,879·78	939·89	3,838·18	1919·09
Sa	1	8·87	8·87	288·19	288·19
Ss	1	2,014·03	2014·03*	23·46	23·46
Ca	1	1,186·28	1186·28	3,549·46	3549·46
Cs	1	632·03	632·03	2,795·03	2795·03
Sa Ss	1	22·00	22·00	904·40	904·40
Sa Ca	1	238·47	238·47	330·43	330·43
Sa Cs	1	86·53	86·53	94·66	94·66
Ss Ca	1	1,895·21	1895·21*	825·37	825·37
Ss Cs	1	1,713·92	1713·92*	2,188·43	2188·43
Ca Cs	1	22·25	22·25	1,831·06	1831·06
Sa Ss Ca	1	18·45	18·45	158·14	158·14
Sa Ss Cs	1	146·94	146·94	225·47	225·47
Sa Ca Cs	1	35·47	35·47	148·74	148·74
Ss Ca Cs	1	11·74	11·74	767·38	767·38
Sa Ss Ca Cs	1	735·31	735·31	1,102·85	1102·85
Experimental error	30	9,532·16	317·79	28,602·78	953·43
Between half-plots	48	14,268·42	297·26	24,620·84	512·93
Within half-plots	1343	96,328·30	71·73	227,333·66	169·27
Total	1438	130,776·16	—	299,628·52	—

Table III.

"Actual" yields ($\frac{1}{4}$ lb. per plot).

Fraction	Degrees of freedom	Grain		Straw	
		Sum of squares	Mean square	Sum of squares	Mean square
Blocks	2	2,083·04	1041·52	5,755·17	2877·58
Sa	1	75·00	75·00	3,960·33	3960·33*
Ss	1	3,780·75	3780·75*	2,730·08	2730·08*
Ca	1	1,365·33	1365·33*	4,485·33	4485·33*
Cs	1	140·08	140·08	3,780·75	3780·75*
Sa Ss	1	261·33	261·33	4·09	4·09
Sa Ca	1	2·09	2·09	16·34	16·34
Sa Cs	1	8·34	8·34	234·09	234·09
Ss Ca	1	1,976·34	1976·34*	5,084·09	5084·09*
Ss Cs	1	720·75	720·75	5,896·34	5896·34*
Ca Cs	1	1,045·34	1045·34	36·75	36·75
Sa Ss Ca	1	420·08	420·08	200·07	200·07
Sa Ss Cs	1	972·00	972·00	456·32	456·32
Sa Ca Cs	1	352·07	352·07	6·74	6·74
Ss Ca Cs	1	40·33	40·33	47·99	47·99
Sa Ss Ca Cs	1	330·75	330·75	1,160·36	1160·36
Experimental error	30	7,934·30	264·48	15,314·83	510·49
Total	47	21,507·92	—	49,169·67	—

the mean squares corresponding with "between half-plots" and "within half-plots" by means of Fisher's "z" test, shows that both for grain and straw the former is the larger by an amount which would not occur merely by chance as often as once in twenty times. It may be concluded, then, that it has been advantageous to divide the plots transversely, and to take half the total number of metre-lengths from each half-plot. For had this not been done, there would have been plots on which the metre-lengths came nearly all from one half; and the accuracy of the yield estimate would have been diminished, since the two halves are shown to differ significantly.

The fraction "within half-plots" represents the variation between metre-lengths of the same half-plot, and provides a direct estimate of the sampling-error, since all variation due to treatment and position of the plot, and to differences between half-plots of the same plot, are here eliminated. The square root of the mean square gives the sampling-error of a single metre-length. Since there are 30 metre-lengths from each plot, the sampling-error of a plot mean is obtained from this by dividing by $\sqrt{30}$.

By treating the "experimental error" mean square in exactly the same way, an estimate is obtained of the variability of the "sampling yield" of a single plot when correction has been made for the average fertility of the block in which it falls, and for the manurial treatment which it has received. It thus includes errors due to sampling as well as those due to differences in fertility between plots of the same block, and to working errors; and is the appropriate basis for determining the significance of manurial effects.

In the case of "actual yields" only one of these quantities can be calculated—the experimental error per plot—and this is simply the square root of the corresponding mean square.

Table IV gives the values of these errors for grain and straw, and for both sets of data.

Table IV.

	Grain			Straw		
	Mean square	Standard error	Standard error per cent. per plot	Mean square	Standard error	Standard error per cent. per plot
(a) "Sampling yields":						
Experimental error	317·74	3·254	12·36	953·43	5·638	13·09
Within half-plots	71·73	1·546	5·87	169·27	2·375	5·51
(b) "Actual yields":						
Experimental error	264·48	16·26 ·	11·07	510·49	22·59	7·90

The sampling-errors are higher than the expected 5 per cent. per plot. This is almost certainly due to winter mortality, many plants being killed by the severe frosts of February, 1928. Despite this the experimental error per plot has been only slightly increased in the case of grain (11·07 to 12·36 per cent.). The increase is more serious for straw (7·90 to 13·09 per cent.): reasons for this will be discussed later.

The real test of the adequacy of a sampling method is the comparison of the amount of information obtained by its use, with that obtained when large-scale methods are employed. Tables II and III give complete analyses of the effect of the various manurial treatments. The test of significance consists in comparing, by means of the "z" distribution, the appropriate mean square with the "experimental error" mean square. Those items which show a significantly higher variation than that due to experimental error (taking odds of 1 in 20 as the level of significance) are marked with an asterisk. It will be seen that, for grain, both sets of data yield three significant items, of which two are common, Ss, and the first order interaction SsCa. The autumn application of cyanamide (Ca), which would be judged effective on the basis of the analysis of "actual yields," just fails to reach the 1 in 20 level in the analysis of "sampling yields"; and the first order interaction of the two spring dressings (SsCs) is significant in the latter but not in the former analysis. Substantially the same results are thus obtained by the two methods: the differences may be due to differences in the mesh of the dressing screens.

For straw the differences are much more striking. While no less than six items are starred in Table III, there are none in Table II (Ca just fails to reach the 1 in 20 level). This is the more curious in that the sampling-error per plot is actually lower for straw (5·51 per cent.) than for grain (5·87 per cent.). Two factors seem to be at work here. In the first place the height above ground at which the straw was cut was constant for the large-scale method, where a binder was used; but varied a little from plot to plot where several different workers were cutting samples. Secondly, the crop was very weedy, owing to the thinness of the plant after the winter frosts, and since all the weeds were included in the "large-scale" sheaves, but were partially discarded from the sampling sheaflet, the yields of straw would be expected to differ on this account. The effect of the first factor would be to increase the experimental error as calculated from the sampling data. This would tend to obscure real effects of manurial treatment. A hint that this surmise is correct is obtained by comparing the difference between the two

estimates of experimental error with the sampling-error. If the only important additional source of variation is the sampling technique, then

$$V_s = V_1 - V_2,$$

where V_s = relative variance due to sampling,

V_1 = relative variance corresponding with experimental error for "sampling" yields,

V_2 = relative variance corresponding with experimental error for "actual" yields.

For the relative variances we may use the squares of the sampling and experimental percentage errors. Then, for grain:

$$V_s = 5.87^2 = 34.46; \quad V_1 - V_2 = 12.36^2 - 11.07^2 = 30.23$$

and the agreement is good.

For straw, however:

$$V_s = 5.51^2 = 30.36; \quad V_1 - V_2 = 13.09^2 - 7.90^2 = 108.94.$$

There is here a considerable difference in the expected direction, supporting the view that the experimental error calculated from the sampling data differs from that calculated from the "actual" yields by another important factor in addition to the sampling-error, this being the variation in the length of straw cut from the different plots.

Support for the second assumption is found in comparing the estimates of average yield and the ratios of straw to grain obtained by the two methods (Tables V and VI).

Table V. *Mean yield in cwt. per acre.*

Grain	"Actual yields"	...	...	13.12
	"Sampling yields"	...	...	13.77
Straw	"Actual yields"	...	...	25.54
	"Sampling yields"	...	...	22.52

Table VI. *Ratio of straw to grain.*

"Actual yields" ...	...	...	...	1.95
"Sampling yields"	...	...	...	1.64

Here the estimates of mean yield of straw differ by more than 12 per cent. in favour of the large-scale method, as would be expected if a considerable quantity of weed were weighed with the sheaves. Further evidence for this view is that the discrepancies between the estimates of straw yield are greater for plots receiving sulphate of ammonia than

for plots receiving cyanamide, a result to be expected if cyanamide depresses the germination of weed seeds.

This is an important conclusion, since the significant effects which emerge from analysis of the "actual yields" are now under suspicion as being probably due in part to differences in amount of weed infestation. The sampling method, it should be noted, provides a means of estimating this disturbing factor on experimental plots, or, alternatively, of eliminating it.

2. ROTHAMSTED BARLEY EXPERIMENT.

The arrangement of this experiment has already been described (p. 367). The important feature from the point of view of the sampling method was that each of the 50 plots was divided into quarters, 1/160th acre in area, which received different manurial treatments. The separate harvesting of these quarter-plots was effected only by the sampling method; the task would have been very difficult or impossible if large-scale machinery had to be employed.

The sampling and experimental errors are shown in Table VII.

Table VII.

	Grain		Straw	
	Whole plot	Quarter-plot	Whole plot	Quarter-plot
A. Square at the lower level of nitrogen.				
(a) "Sampling yields":				
Experimental error	5·58	13·70	13·56	14·99
Sampling-error	5·53	11·07	5·58	11·17
(b) "Actual yields":				
Experimental error	8·46	—	10·04	—
B. Square at the higher level of nitrogen.				
(a) "Sampling yields":				
Experimental error	6·98	10·64	7·58	11·71
Sampling-error	4·99	9·97	5·09	10·19
(b) "Actual yields":				
Experimental error	3·77	—	6·26	—

The sampling-errors for whole plots are those of means of 32 sampling-units, and are comparable with the values obtained in the winter oats experiment, 5·87 per cent. for grain and 5·51 per cent. for straw. It will be seen that the expected value of about 5 per cent. was obtained in plots which received the double quantity of nitrogen. It is almost invariably found, as here, that an area bearing a heavy crop gives smaller experimental errors than one otherwise similar but with a light crop. The fact that the sampling-errors are also smaller suggests that the ex-

planation lies in the greater capacity of a heavily manured crop to compensate for unevenness in the original plant. In a cereal crop this compensation usually takes the form of an increased number of ear-bearing tillers on the plants adjoining gaps.

The very small experimental error for "actual yields," especially of grain, in plots receiving the heavy dressing, exaggerates the difference between the two methods of harvesting. An error as low as 3·77 per cent. must be regarded as exceptional, however; usually the plot error falls between 6 and 12 per cent. of the mean yield.

Only eight sampling-units were taken from each quarter-plot, and the sampling-errors are double those for whole plots.

Table VIII. *"Sampling yields" (gm. per quarter-plot).*

Fraction	Degrees of freedom	Grain		Straw	
		Sum of squares	Mean square	Sum of squares	Mean square
A. Square at lower level of nitrogen.					
Rows	4	9,787·69	2,446·92	19,456·86	4,864·22
Columns	4	55,412·72	13,853·18*	55,328·13	13,832·03
Treatments	4	45,993·74	11,498·44*	66,126·06	16,531·52
Error (a)	12	18,146·00	1,534·67	112,909·43	9,409·12
P	1	0·81	0·81	1·32	1·32
K	1	306·25	306·25	95·06	95·06
PK	1	169·00	169·00	1,447·80	1,447·80
Nit. × P	1	122·10	122·10	666·93	666·93
Nit. × K	1	473·06	473·06	405·02	405·02
Nit. × PK	1	5,076·56	5,076·56	7,881·00	7,881·00
Qual. × P	3	10,417·34	3,472·45	9,069·98	3,023·33
Qual. × K	3	13,382·64	4,460·88	15,520·21	5,173·40
Qual. × PK	3	11,042·24	3,680·75	16,495·53	5,498·51
Error (b)	60	138,730·75	2,312·18	172,364·60	2,872·74
Total	99	355,054·64	—	477,767·93	—
B. Square at higher level of nitrogen.					
Rows	4	43,989·12	10,997·28*	121,172·74	30,293·18*
Columns	4	5,816·61	1,454·15	27,418·91	6,854·73
Treatments	4	27,489·22	6,872·31	77,191·89	19,297·97*
Error (a)	12	38,643·32	3,220·28	46,642·55	3,886·88
P	1	53·29	53·29	64·00	64·00
K	1	14,328·09	14,328·09*	15,951·69	15,951·69*
PK	1	11·56	11·56	234·09	234·09
Qual. × P	4	9,405·79	2,351·45	14,122·78	3,530·70
Qual. × K	4	9,036·79	2,259·20	18,242·49	4,560·62
Qual. × PK	4	4,550·92	1,137·73	5,434·24	1,358·56
Error (b)	60	112,254·93	1,870·92	139,255·08	2,320·92
Total	99	265,579·64	—	465,730·46	—

Error (a) is the basis for direct comparison of whole-plot treatments, and error (b) for quarter-plot treatments and their interactions with the whole-plot (nitrogenous) treatments. Nit. × P, etc. are interactions of the quarter-plot treatments with nitrogen, irrespective of the form in which the nitrogen is applied. Qual. × P, etc. are differential responses to the quarter-plot treatments on plots receiving nitrogen in different forms.

The analyses of variance for "sampling yields" and "actual yields" are given in Tables VIII and IX, fractions significantly exceeding that ascribable to experimental error being marked with an asterisk.

Table IX. *Actual yields ($\frac{1}{4}$ lb. per plot).*

Fraction	Degrees of freedom	Grain		Straw	
		Sum of squares	Mean square	Sum of squares	Mean square
A. Square at lower level of nitrogen.					
Rows	4	2,205·04	551·26	1,533·04	383·26
Columns	4	2,161·84	540·46	8,194·24	2048·56*
Treatments	4	10,175·44	2543·86*	13,073·84	3268·46*
Error	12	5,808·52	484·04	8,387·92	698·99
Total	24	20,351·84	—	31,189·04	—
B. Square at higher level of nitrogen.					
Rows	4	5,519·04	1379·76*	7,429·60	1857·40*
Columns	4	6,080·24	1520·06*	3,246·80	811·70
Treatments	4	2,668·24	667·06*	3,865·20	966·30*
Error	12	1,463·92	121·99	3,838·40	319·87
Total	24	15,731·44	—	18,380·00	—

It will be seen that whole-plot treatments appear effective in all cases when "actual yields" are analysed, but that "sampling yields" fail to show an effect on straw at the lower level and on grain at the higher level of nitrogen. These results are shown in the table of percentage yields (Table X).

Table X.

A. At lower level of nitrogen.

Grain	O	S	M	N	C	Mean	Standard error	Mean (cwt. p. a.)
Actual	86·4	99·6	101·6	110·5	101·9	100·0	3·79	23·21
Sampling	90·2	103·1	102·7	107·7	96·3	100·0	2·50	22·93
Straw								
Actual	86·4	101·6	98·3	113·7	100·0	100·0	4·49	23·51
Sampling	88·4	101·6	103·2	110·1	96·7	100·0	6·06	23·37

B. At higher level of nitrogen.

Grain	U	S	M	N	C	Mean	Standard error	Mean (cwt. p. a.)
Actual	97·0	96·3	100·0	106·3	100·4	100·0	1·68	26·19
Sampling	93·7	98·0	99·5	105·1	103·6	100·0	3·12	26·57
Straw								
Actual	94·3	97·7	100·0	107·5	100·6	100·0	2·80	25·50
Sampling	94·0	95·1	94·6	106·3	110·0	100·0	3·39	26·89

O =no nitrogen; S =sulphate of ammonia; M =muriate of ammonia; C =cyanamide; N =nitrate of soda; U =urea.

The information lost is in each case the significant superiority of nitrate of soda to other sources of nitrogen, found in all cases for "actual

yields," but only for grain at the lower level for "sampling yields." At the higher level of nitrogen the "sampling yields" of straw show both cyanamide and nitrate of soda significantly above the other three forms of nitrogen. This difference in the position of cyanamide is curious, but is perhaps due to the partial removal of weeds from sampling sheaflets, as in the oats experiment. It is frequently claimed for cyanamide that it inhibits the germination of weed seeds: if there were a real lessening of the weight of weeds on plots treated with this fertiliser, the effect would be that observed.

The sampling method is shown to better advantage in the quarter-plot results. It will be remembered that the quarter-plots were only 1/160th acre in area, and could hardly have been dealt with by large-scale methods. The quarter-plot treatments were identical for each whole plot: (1) no additional treatment; (2) superphosphate; (3) sulphate of potash; (4) both superphosphate and sulphate of potash: the allocation of the four treatments to the quarter-plots within any plot was at random. The analyses of Table IX show that neither phosphate nor potash was effective at the lower level of nitrogen, but that at the higher level there was a significant response to potash both in grain and straw. As Table XI shows, this response was a depression in yield. Its magnitude was quite small—5·88 per cent. for grain and 6·14 per cent. for straw—but the low standard error makes even so small a difference significant. This is a striking demonstration of the efficiency of the experimental arrangement, as well as an example of the manner in which sampling can act as a valuable auxiliary to large-scale harvesting methods.

No differential responses to potash or phosphate on plots bearing different forms of nitrogen were detected, as is shown in the analyses.

Table XI.

	O	P	K	PK	Mean	Standard error
	A. At lower level of nitrogen (percentage of mean yield).					
Grain	100·84	100·15	99·11	99·90	100·00	2·74
Straw	100·82	98·63	99·24	101·30	100·00	3·00
	B. At higher level of nitrogen.					
Grain	102·85	103·04	96·79	97·32	100·00	2·13
	102·94		97·06			
Straw	102·50	103·64	97·11	96·75	100·00	2·34
	103·07		96·93			

3. ROTHAMSTED WHEAT EXPERIMENT.

The wheat experiment of 1928–9 was designed to give information as to the effect of applying sulphate and muriate of ammonia as top dressings to four different varieties of wheat. The top dressings were given either early (March 18), late (May 13), or at both these dates. Owing to the severe frosts of February and March 1929 the plant was very thin, and later the plots became infested with Black Bent (*Alopecurus agrostis*). The weediness was much more marked at one side of the experimental area than at the other, and tended to increase still further what must in any case have been a large experimental error. As a result no treatment or variety differences could be regarded as significant, and no more information was obtained by the large-scale than by the sampling method. Table XII gives the sampling and experimental errors per plot, expressed as percentages of the mean yield.

Twenty-four metre-lengths of drill were cut from each of the plots. The area of each plot was 1/55th acre.

Table XII.

	Grain		Straw	
	(a)	(b)	(a)	(b)
(a) "Sampling yields":				
Experimental error	11·69	20·39	14·41	16·89
Sampling-error		5·84		6·73
(b) "Actual yields":				
Experimental error	9·68	22·48	9·99	15·14

Columns (a) and (b) show the plot errors for varietal and treatment comparisons respectively.

4. WELLINGORE BARLEY EXPERIMENT.

By courtesy of G. H. Nevile, Esq., of Wellingore Hall, Lincs., an experiment was carried out on Lincoln Heath, near the village of Wellingore. This consisted of sixteen plots, bearing eight different treatments in duplicate—no artificial fertilisers, and any one, two, or three of the following dressings: sulphate of ammonia at 1 cwt. per acre, sulphate of potash at 1 cwt. per acre, and superphosphate at 3 cwts. per acre. The plots were each 1/60th acre, and were harvested only by a sampling method. Forty half-metre lengths of drill were cut from each plot, but actually there were only four sampling units—*i.e.* four independently located parts of the sample (2). The procedure was to select four drill-rows at random from each plot (discarding edge-rows), and

then to cut 10 half-metre-lengths as shown in the diagram (Fig. 1). The measuring-rod was that used at Rothamsted, 2 half-metres being separated by a metre.

A constant number of paces separated successive placings of the rod. It is readily seen that this method gives a complex sampling-unit which involves four rows, and that four such sampling-units must provide a satisfactorily representative sample of the produce of the plot.
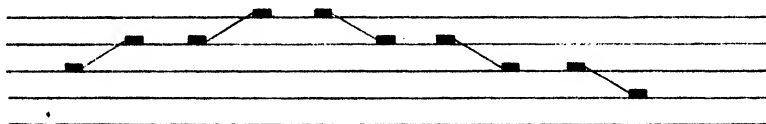
Fig. 1.

The analyses of variance for grain and straw are shown in Table XIII, fractions significantly exceeding that ascribable to experimental error being marked with an asterisk.

Table XIII.

Fraction	Degrees of freedom	Grain		Straw	
		Sum of squares	Mean square	Sum of squares	Mean square
Blocks	1	6,172·07	6,172·07	744·27	744·27
K	1	2,217·82	2,217·82	2,960·04	2,960·04*
P	1	962·94	962·94	1,567·67	1,567·67*
N	1	17,797·23	17,797·23*	29,745·47	29,745·47*
PK	1	199·52	199·52	345·73	345·73
NK	1	826·56	826·56	2,629·77	2,629·77*
NP	1	14,475·10	14,475·10*	11,469·07	11,469·07*
NPK	1	2,044·73	2,044·73	2,604·18	2,604·18*
Error	7	4,551·94	650·28	2,021·19	288·74
Within plots	48	22,936·90	477·85	22,740·95	473·77
Total	63	72,184·81	—	76,828·34	—

The sampling-error per plot was 5·31 per cent. for grain, and 5·78 per cent. for straw; and the experimental errors 6·19 per cent. and 4·51 per cent. respectively.

It is interesting to note that for grain two, and for straw no less than six of the treatment items were found to be significant. The low experimental errors which make the experiment so useful can doubtless be ascribed to the exceptional uniformity of the soil and the plant. That the plant was uniform is further shown in the magnitude of the sampling-errors, which are of the same order as in Rothamsted experiments where 30 or more metre-lengths were taken from each plot.

<div align="center">DISCUSSION.</div>

The results are summarised in Table XIV. The experiments with barley certainly justify the claims made in an earlier paper for the accuracy and usefulness of the sampling method described. The sampling-error per plot has been rather more than 5 per cent. of the mean yield; the experimental error, as for grain at the lower level of nitrogen in the barley experiment, may actually be lower than the corresponding large-scale figure; little information has been lost which a large-scale method would have given; plots were successfully dealt with which would have been much too small for large-scale experimentation; and the large-scale methods were entirely dispensed with in an outside experiment which yielded a great deal of information as to the effects of various fertiliser combinations. Further advantages are that edge-rows can be discarded without the necessity of removing them; losses in the stook and in the stack are avoided; results are available sooner than would normally be the case with stacked corn; and the bulked produce of the independently located sampling-units constitutes an excellent sample for analytical work.

<div align="center">Table XIV.</div>

Crop	Size of sample (metres)	Area of plot (acre)	Sampling-error (% per plot)	Experimental error (% per plot)	
				(a) "Sampling yields"	(b) "Actual yields"
Wheat:					
1. Grain	24	1/55th	5·84	11·69, 20·39	9·68, 22·48
2. Straw			6·73	14·41, 16·89	9·99, 15·14
Oats:					
1. Grain	30	1/40th	5·87	12·36	11·07
2. Straw			5·51	13·11	7·90
Barley:					
(a) 1. Grain	32	1/40th	5·53	5·58	8·46
2. Straw			5·58	13·56	10·04
(b) 1. Grain	32	1/40th	4·99	6·98	3·77
2. Straw			5·09	7·58	6·26
Barley (Wellingore):					
1. Grain	20	1/60th	5·31	6·23	—
2. Straw			5·78	4·51	—

The experiments with winter-sown cereals were somewhat less pleasing, but the higher sampling-error per plot can almost certainly be ascribed to the depletion of plant by the severe frosts of the winter 1928-9. Little useful information was derived from the wheat experiment. From the results of the oats experiment, however, it is shown that the sampling method can be used to give the weight of straw freed from weeds, and also an estimate of the effect of various fertilisers on weed growth.

Where large-scale equipment is already in use it could hardly be suggested that this should be entirely replaced by the apparatus necessary for the sampling method. The results of the 1929 experiments show, however, that sampling for yield might well be adopted as an auxiliary method, and where no large-scale machinery is already available it would further recommend itself through the relative cheapness of the necessary equipment. It solves the problem of harvesting complex experiments on farms at some distance from the organising station, and by thus permitting the repetition of experiments on many types of soil, greatly enhances their value.

The practicability of dealing with small plots is an important point. It has been shown by Roemer(4) and others that for a given experimental area, to be used for the comparison of a given number of varieties or treatments, it is of much greater advantage to increase the number of replications than to increase the size of the individual plot. In other words, the loss of accuracy arising from reduction in the size of the individual plot is more than counterbalanced by the gain from a higher degree of replication. The labour of sampling, however, from the experimental area may not be greatly increased by an increase in the number of plots into which it is divided, and the absolute size of the individual plot does not in any way affect the practicability of sampling. The extent to which the total size of sample taken from the area is altered depends, of course, on the nature of the variations in yield per unit length of drill over the area. Thus if the mean yield of a small plot (for constant treatment), and the variability within the plot, were fairly constant throughout, it would be necessary to take almost as many sampling-units from a small plot as from a large plot. If, on the other hand, mean fertility varied considerably between small plots, it would be possible to reduce the number of sampling-units when the plot-size is reduced. It may be said in general that the number of sampling-units to be taken from a small plot can be at least $(n-1)$ less than the number taken from a larger plot, where the areas are in the ratio $1:n$, provided that it proved profitable to subdivide the larger plot into n parts for the purpose of sampling. The test of the advantage gained by subdivision is the significance of the difference between the mean squares for "within subdivisions" and "between subdivisions," as explained on p. 379.

It may be noted that it is not essential to take a large number of sampling-units from each plot, though in exploratory work such as that described it was desirable in order to obtain an accurate estimate of the sampling-error, of the advantage gained by subdivision of plots, etc. When such preliminary work has been completed, it should be sufficient

to take two or three complex sampling-units from each plot, the size of the sample remaining, of course, unchanged.

In conclusion it should be pointed out that for convenience in sampling the distance between drills should be not less than 7 in., and that where choice is possible, a stiff-strawed variety should be grown, since lodged corn is very difficult to sample adequately.

SUMMARY.

1. Four cereal experiments, comprising 210 plots each about 1/40th acre in area, were harvested by a sampling method. Three of the experiments were later harvested by large-scale methods, so that a direct comparison could be made.

2. The field technique is described, and an account is given of the small combined thresher and winnower which was constructed for the purpose of dealing rapidly with the numerous small sheaflets.

3. The results are analysed in detail and it is shown that the sampling-errors per plot lie between 5 and 6 per cent. of the mean yield, and that these errors are sufficiently low for there to be little loss of information.

4. The relative advantages of large-scale and sampling methods are discussed, with special reference to the possibility of dealing with large numbers of very small plots, and of carrying out complex experiments on farms distant from the organising station.

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INVESTIGATIONS ON YIELD IN THE CEREALS. VICTORIA. I.

CENSUS STUDIES 1927–9.

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(With Two Text-figures.)

INTRODUCTION.

THE intensive study of cereal crop growth has been a subject of great interest in England during the last few years. Keen competition and the gradual falling of the price level have stimulated the need for a detailed knowledge of plant behaviour. In response to this need, a new method was developed in 1922 for the observation of the growing crop (1, 2). It consisted of the actual counting of the numbers of plants in certain definite samples of 1 ft. length of drill row, scattered over the area in a systematic manner. In subsequent counts a record was made of the development of these plants through their various growth periods until harvest.

Australia, at present economically dependent on primary production, is concerned in no less degree with the maximum economic increase in the yield of her cereal crops. Various major improvements in wheat culture have increased yields considerably in the past—in cultural practices by "fallowing," in manuring by the use of superphosphate, and in the breeding and selection of new and improved varieties. The definite rise in the average yield of the Wimmera district of Victoria from 6 to 7 bushels per acre in 1892–6 to an average of 22 bushels per acre from 1922–5 is an instance of the results achieved. Further improvements demand a more intimate knowledge of crop behaviour which should light the plant breeder's path in his attempts at the production of still better varieties. Yield trials have shown the adaptability of some varieties to certain districts, and the definition of the underlying reasons for these facts should enable him to build up improved strains.

The method of census study developed in England was particularly suited for this purpose, and thus the Department of Agriculture of

Victoria in 1927 commenced a series of observations on crop growth along the lines laid down by the English workers. Areas of crop in the various representative districts of the State were examined by a method of sampling similar to that used by Engledow[1]. As a result of the particular conditions of crop growth, usually only three counts were made each season, (*a*) at germination, (*b*) for the maximum tillering, and (*c*) at harvest. On each area studied, therefore, the following information was obtained from the respective observations:

(*a*) Germination count:
  (1) numbers of plants per foot at germination.

(*b*) Tillering count:
  (1) numbers of plants per foot in early spring,
  (2) numbers of tillers produced per plant.

(*c*) Harvest count:
  (1) numbers of plants per foot at harvest,
  (2) numbers of ears per plant,
  (3) numbers of grains and spikelets per ear,
  (4) average weight of a single grain.

During the first few years, the four most important varieties were studied in each district, but so useful did the information obtained appear that these methods have been extended to other types of trial, and it is now the practice to conduct a census investigation in conjunction with every important yield trial.

It has been found, as might be expected, that the type of result obtained has differed materially from the English figures, and therefore the presentation of the problems confronting Australian workers may be of wider interest.

CLIMATIC CONSIDERATIONS[1].

In a general way the wheat zones of Australia may be grouped into three classes:

(*a*) The sub-tropical areas (the north of New South Wales, Queensland), in which the plants develop mainly on the residue of the summer and autumn rains. It is essential that the plants should mature by November before rust develops to the detriment of the crop and before summer rains make harvesting difficult.

(*b*) The dry temperate zone (Southern N.S.W., Victoria, South Australia, and Western Australia), which area contributes the greatest

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[1] For complete meteorological data see (9).

portion of the wheat crop. Rainfall is from $7\frac{1}{4}$ to 20 in. per annum with a winter maximum.

(c) The wet temperate zone (highland portions of Victoria and Tasmania), comprising areas having an average rainfall of more than 20 in., with conditions more nearly approaching those in England.

It is with the second zone that the present studies deal.

The census observations were conducted in four districts of Victoria, and it is necessary to give a short résumé of the conditions prevailing in these areas.

(1) The Mallee district—comprising on the average 47 per cent. of the total area sown to wheat in Victoria. The locality studied was at Carwarp, which is a rather extreme case for the Mallee as a whole. The annual yield here averages 9–10 bushels per acre, indicating the "marginal" nature of portions of this area on account of the marked uncertainty of its rainfall, which averages about 11 in. per annum and falls chiefly in the winter months. The soil is a sandy loam of very easy texture and capable with moisture of supporting a very vigorous vegetation.

(2) The Wimmera—locality, Longerenong. The areas studied here are on typical black, self-mulching soil of the "black earth" type (Prescott). These soils are of high fertility and capable, in favourable seasons, of yielding up to 50–60 bushels per acre. Their average is about 30 bushels per acre. In other portions of the same district, reddish loams and clay loams predominate. Rainfall is about 12–16 in. per annum and is fairly constant with a winter maximum. The area contributes about 25 per cent. of the wheat acreage of Victoria at the present time.

(3) The North-east—locality, Rutherglen—situate on the foot hills of the dividing range. The average rainfall is 21 in. per annum with a high winter maximum. It approaches in normal seasons to the type of zone (c) mentioned above (*supra*), but is earlier. The soil is of a silty nature and may set like cement after heavy rain.

(4) The Central district—locality, Werribee—typically a rather heavy clay loam derived from Quaternary Basalt tending to set after heavy rains; it is subject to very heavy winds which probably considerably decrease the value of the 19 in. rainfall, a large portion of which falls as light showers.

The seasonal conditions during 1927–8–9 have been decidedly dry, with rainfalls below average in all the districts studied. The year 1928 opened very favourably with rather heavy autumn rains and the crops showed fine development, but a dry period in August and September

caused a severe set-back to all plant development especially in the Mallee areas, and the crops generally failed to finish as they had promised.

The year 1929 was more unfortunate still for the northern areas since the very light autumn and early winter rains failed even to germinate the grain at Carwarp. A total loss of crop was thus recorded. In the Wimmera rains were sufficient for normal tiller development, and later
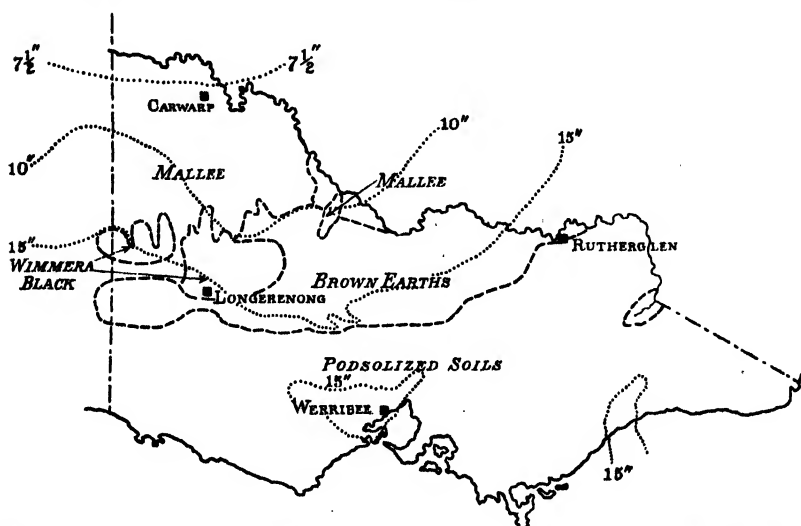
Map of Victoria showing Isohyets and Soil Zones. (Kindly supplied by Professor Prescott.)
The Isohyets indicate the average "winter" rainfall from April to November.

failed to mature an average crop. These conditions of subnormal rainfall enabled the North-eastern district, which often suffers from water-logging in the winter months, to develop yields above their average during the three seasons. The Central district was also more fortunate during 1929 and matured good crops in that year.

CULTURAL PRACTICES AND CROP GROWTH.

Crop growth under these conditions of light rainfall is likely to be of a fundamentally different nature from that under English climatic conditions.

Seeding is usually delayed until the first autumn rains have fallen, in March or April. The land has generally undergone a period of fallowing up to 14 months in extent, during which time the surface has been kept more or less cultivated and the weeds eliminated as far as possible. A light cultivation is thus all that is necessary immediately prior to

seeding. The grain is sown by means of a seed drill in much the same manner as is customary in England, at rates which vary from 50 to 60 lb. per acre in the Mallee to 90 lb. in the Central district. One important variation is the accompanying application through a rear portion of the drill of superphosphate in quantities ranging from 70 to 100 lb. in the Mallee and up to 1½–2 cwt. per acre in the moister portions of the State. By means of a system of spouts, the superphosphate is placed alongside the seed as it falls into the furrow. Before seeding, farmers are advised

Diagram showing crop development periods.

S Seeding.
T Completion of tillering.
H Heading period.
R Harvesting period.
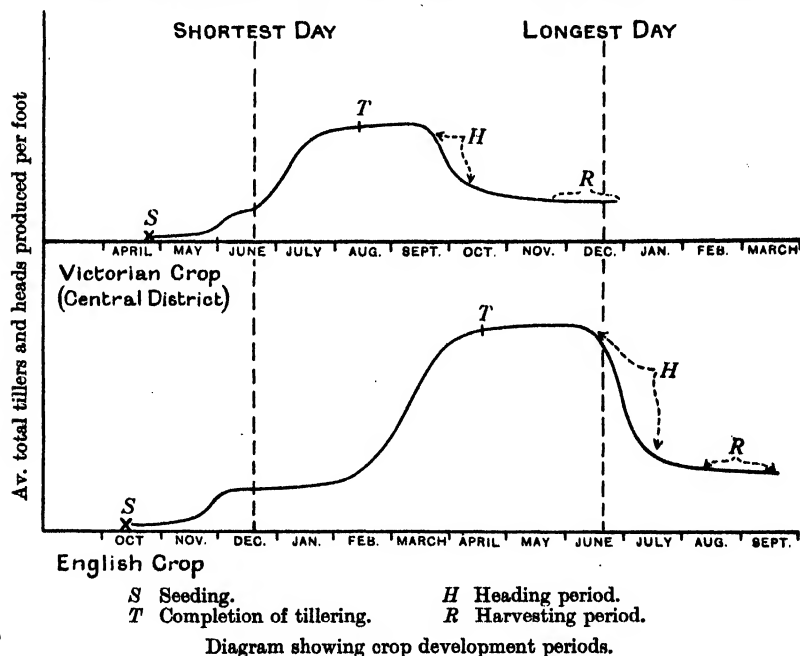
to dust all grain with dry copper carbonate powder as a preventative for Ball Smut disease (*Tilletia tritici*).

The average temperature of the soil during the winter months[1] is sufficiently high to allow growth to proceed at all periods. Consequently, after germination in May, but a short period (3–4 weeks) elapses before tillering occurs, accompanied by the formation of flower primordia. In this manner that long period of "over-wintering" in the "rosette" stage, with its attendant dangers to plant population, is avoided.

Tillering is generally completed by the end of August and the heads

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[1] Werribee has a mean average temperature of soil at 1 in. of 48·7° F. during the two coldest months, June and July.

emerge some four months after germination. The subsequent history of the plants depends entirely on the incidence of the rainfall in the later months, and thus it has been found that both size of head and, to a less extent, weight of grain vary with the season.

The final stage of harvesting is completed by a machine, either a "harvester" or a "header," which combines in one single operation the three processes of stripping or cutting the heads from the straw as it stands in the field, thrashing the heads and winnowing the grain. It is this reduction of handling costs at harvest that enables the farmer to produce more cheaply than his English competitor.

<center>YIELD FACTORS.</center>

Turning now to the observations on the behaviour of the various areas studied, the standard components of yield, p = population, e = ears produced per plant, n = number of grains per ear, g = weight of grain, will be considered in turn.

Population, the "p" factor. The marked variation in the spacing of the plant populations, so much a feature of English observations, appears to be equally noticeable under Australian conditions. Numerous "makes" of drill have been tested under varying conditions and in no case was the distribution of seed at all constant. The "coefficient of variation" for number of plants per foot of row ranged from 20 to 40 per cent., according to the class of seed bed. The Wimmera soils, on account of their fine texture, afforded the least variation.

The practice of implement manufacturers in Australia is to space the coulter hoes 7 in. apart as against the 8 in. spacing of English drills quoted by Engledow. Although the reason behind the 7 in. spacing is indefinite, its use in practice has proved satisfactory. However, since the 7 in. spacing is in vogue in Australia, this fact must be remembered when comparing populations per foot of drill row. With Australian drills there are approximately 74,700 ft. of drill row per acre, with English drills 65,400; so that, while a seeding of 120 lb. per acre of normal-sized grain would drop an average of 16·0 seeds per foot in the former case, a similar seeding in England would give a population of 18·3 seeds per foot.

One most important factor in the production of the higher English wheat yields per acre is the large plant population which ranges about 14–16 plants per foot, and is derived from rates of seeding of 2–3 bushels per acre. All Victorian rate-of-seeding trials indicate that the maximum

density of crop which can be supported by our rainfall conditions will be derived from a seeding ranging from approximately 8 seeds per foot in the dry areas of the Mallee to about 12 seeds per foot in the more southern districts. Thus there appear, right at the commencement of these studies, the salient features of wheat culture in Victoria; firstly the need to conserve all available moisture, secondly the need to restrict the plant population to an amount which can be supported to harvest (and thirdly, as will be shown later, the necessity for varieties which are drought resistant). All these features are but variants of that one main limiting factor of available moisture for plant growth.

Any history of plant development under English conditions is concerned with the importance of obtaining good germination and survival of plants over the severe winter conditions during the first few months after seeding, when the plants generally suffer from "winter sickness" or "yellowing." While, in general, Australian germination figures under field conditions range over the same values, plants in Victoria have little difficulty in surviving the winter. Under normal conditions, about 95 per cent. of the plants counted at germination survive till harvest. Disease and droughty conditions may considerably lower the number of plants maturing grain, as the following figures indicate.

Table I. *Showing, for the districts concerned, the percentage survival of plants from germination to harvest. (Average figures for varieties studied.)*

	1928	1929
Percentage of germination:		
Mallee	80	Nil
Wimmera	73	91
North-east	77	80
Central	62	74
Percentage of plants surviving from germination and producing heads:		
Mallee	96	—
Wimmera	96	97
North-east	94	97
Central	88	91
Percentage of plants at harvest *failing* to produce grain:		
Mallee	25	—
Wimmera	5	27
North-east	11	5
Central	23	7

The Mallee during 1929, owing to the entire lack of autumn and early winter rains, afforded an example of one of the few cases of total failure of crop. The seed was sown in April on a dry seed bed in the normal manner, but failed to appear to any appreciable extent either then or later in the year. The figures for the Central district show the lowest

values both for germination and percentage survival, on account of the clayey nature of the soil and the colder conditions experienced through the winter. During wet seasons in the North-east, trouble of a similar nature to English "yellowing" might be expected, but this has not occurred during the period under detailed observation.

The high values for loss of "effective" plants during late stages of growth in the Mallee during 1928 (25 per cent.), and in the Wimmera in 1929 (27 per cent.), were due to actual death of plants after heading, mainly through drought and hot winds. The loss of plants in the North-east in 1928 (11 per cent.) was due to an attack of rust, while the corresponding figure for the Central district in the same year (23 per cent.) was due to a very bad attack of Foot-rot (chiefly *Fusarium* spp.).

Tillering and heading, the "e" factor. Turning next to the vegetative growth of the plants, early tillering is a feature of Victorian varieties of wheat. Within 2–3 weeks of seeding in May, the majority of those grains that will germinate have appeared above ground. Two normal leaves soon appear, and in about 6 weeks from seeding the plants have produced shoots or tillers corresponding to the three first leaves of the main axis. This development of what may be called the "primary wheat plant" appears to be a characteristic of Australian varieties, since tillering takes place to this extent at least, regardless of variety, spacing, soil or season, provided that conditions are in any way moderate.

Observations on the development of head primordia show that the "rosette" stage, so characteristic of English winter-sown wheat, is cut down to a minimum. Barely have the tillers time to strengthen before they elongate and begin to shoot upwards. Studies on the effect of varying "lengths of day" have shown that even Victorian late-season wheats have a shorter life history than some of the earliest English crossbreds. This fact indicates that they respond to shorter "lengths of day," or in other words, that, all unconsciously, plant breeders in Australia have been selecting those variant types of the original importations which commence their head formation in response to short winter days. This early development is further encouraged by the fact that the shortest winter days of Victoria (of approximately 10 hours' duration in Melbourne) are considerably longer than the shortest days in England (approximately 8½ hours at Greenwich). In this manner, Victorian varieties are enabled to mature before the dry months of the summer affect them unduly.

By the end of August, tillering has reached finality except under

abnormal conditions, and towards October the heads emerge. Marked variation has been noted in the degree of final tillering with soil and season, with variety, and with spacing and manuring.

Typical district variations in stem production indicate that the total number of tillers per plant under good growth conditions does not show marked difference from the English figures. It must be borne in mind, however, that the population is markedly less. Had the number of plants per foot been in the neighbourhood of 14–16, much smaller values would have been recorded for tillers per plant. From this point of view, tillers per foot of drill row give a better indication of the density of the crop.

Table II. *Showing the tillering and heading for the various districts during* 1928–30.

Variety—Federation.

	Av. tillers per plant	Av. tillers per foot	Av. ears per plant	Av. ears per foot
Mallee	3·60	25·2	1·19	6·7
Wimmera	4·76	48·7	1·90	17·0
North-east	3·84	36·2	1·39	13·4
Central	3·38	29·0	1·48	11·0
English crop (Yeoman)*	4·60	58·4	1·44	18·7

* *Ex* Engledow (5).

The possible yields of the Wimmera, as indicated by heads per foot, thus approach those for English crops, but apart from these soils, tillering and resultant ear production fall away rapidly. The low figure for number of heads per foot in the Mallee indicates the drastic reduction in numbers of plants when dry conditions prevail.

Perhaps the most interesting information on vegetative growth has been obtained from the varietal studies. In the early days, wheat grain for seeding purposes was imported from England and the Continent. Since then these strains have been subjected to many years of natural and artificial selection. Many importations from other countries have supplied the additional basic material on which the plant breeder has worked, until at the present time, the most popular varieties of wheat show a fundamentally different type of growth from that exhibited by the older strains and the related English forms. Not only are the new varieties earlier in maturity (in that they respond to shorter "lengths of day"), but when grown under similar conditions they exhibit firstly, a lower tillering, and secondly, shorter and stronger tillers with but little flag surrounding the base. In the majority of cases, the plants tend to concentrate on the first three main tillers that are formed, and there is

a marked reduction in the number of secondary side tillers. This indicates that there is a tendency towards a conservative type of variety which will concentrate its growth on fewer tillers and will be able to carry these through in spite of deficiency of moisture.

Not only is this tendency exhibited in growth of varieties, but the Victorian practice of low manuring rate tends toward a restricted growth in the spring. Numerous observations of the failure of crops towards harvest have forced farmers to the conclusion that it is essential that the spring growth must be within the limits of what can be carried through to harvest on the average rainfall. The foundations must not be laid in September for a bigger crop than the later average rainfall can mature.

Not only must the spring tillering be conservative, but there is also the need for the utmost use of this growth in head production. This factor, which may be expressed as the "Percentage Survival" of tillers to heads, has been of the greatest importance in indicating drought resistance. All the outstanding new varieties in general cultivation show a marked increase in the value of this factor.

At the present time, those varieties which have been observed in this trial have been grouped into three classes according to their behaviour in respect of heading. The original class from which these presumably have been derived is also included as a fourth.

(*a*) Original types—which are very little removed from the old English strains. The variety, Purple Straw, may be quoted as an example. Very high tillering is shown and the growth is typically sappy and weak, with a low survival of tillers to heads.

(*b*) High-tillering types—varieties Federation and Wannon. These represent the first step away from class (*a*). They were produced by an outcross of "Indian blood." Yield trials show that these varieties are admirably suited for the Wimmera soils under normal seasonal conditions. Wannon, a selection of Federation made during a period of good seasons in the Wimmera, shows an increase in tillering over that of Federation. It also has an improved size of head.

(*c*) Medium-tillering types—Free Gallipoli, Ranee, Nizam. These types represent the second stage away from the original varieties. They have two or more strains of "Indian blood" in them, and show a definitely lower tillering capacity than Federation and in all cases a higher "percentage survival" of tillers to heads. Yield trials show that these varieties are suited to the general wheat belt of Victoria. Even in the Wimmera district during the past dry seasons, these varieties

have outyielded the Federation-Wannon group. The very bulk produced by the latter varieties, the cause of their success in a good season, was their downfall in the dry years. The following figures demonstrate their failure in the past two years.

Table III. *Showing for two varieties the numbers of tillers and heads produced in the Wimmera*, 1928, 1929.

		Tillers per foot	Ears per foot	"Percentage Survival"
1928	Wannon	52·6	16·6	31·6
	Free Gallipoli	37·6	17·9	47·6
1929*	Wannon	61·1	9·45	15·5
	Free Gallipoli	44·9	11·1	24·7

* Very dry finishing conditions.

Both Ranee and Nizam show a percentage survival even higher than Free Gallipoli, and they afford very promising material for breeding in this direction. At present they both suffer from a rather small head weight.

(*d*) Low-tillering types—variety Rajah. These are invariably the very early wheats whose physiology is such that under normal conditions of seeding they barely form the three main primary tillers per plant. Their survival under these conditions is high. They are drought resisting from two points of view:

(1) because of earliness, as they mature before the dry months of November and December can affect them;

(2) because of the economy of growth resulting from the low tillering and high survival of tillers to heads.

As a necessary result, the yields of these varieties are strictly limited. Under normal conditions they seldom have the groundwork for big yields, but have a reliable small yield even in drought periods. In this respect they differ from group (*c*), which although conservative in tillering, nevertheless may, should the season allow, produce sufficient heads to give a good yield. The high-tillering group give the greatest yield under good conditions of soil and climate, but suffer the most in years of drought.

Ear size "n," and grain weight "g." Apart from the varying density of crop, it is in this factor of number of grains per ear that Australian crops appear to suffer in comparison with English ones. The characteristics of the long English head of wheat with its three or more grains across spikelets appear to have been lost under Victorian conditions.

Whether this is a feature of the new varieties, or whether it is in consequence of our drier conditions with perhaps a deficiency in some constituent of our soils, remains to be seen. It would be interesting to determine in which particular the English crops excel, in numbers of spikelets per head or in grains per spikelet. The range of conditions of head size produced may be seen from the table.

Table IV. *Showing a comparison of English and Australian crops as regards ear size and grain weight.*

	Weight of head (gm.)	No. of grains per head	Weight of grain (gm. per 1000 grains)
Good Head (North-east, 1927) var. Free Gallipoli	0·97	23·7	41·0
Average (Mallee, 1928) var. Rajah	0·74	22·0	33·6
Poor Head (Wimmera, 1929) var. Free Gallipoli	0·39	10·5	37·3
English Head (Yeoman)*	1·14	27·8	40·9

* Engledow (5).

The importance of the number of fertile spikelets bearing grain has been stressed in the Victorian studies. The prevalence of sterile spikelets both at the base and at the tip of the ear has been a prominent feature during the past years. Hot winds in October have probably been responsible for the "tipping" of the head, but there may be possibilities of developing the four or five sterile spikelets which are generally present at the base of the head.

The observations have shown that varieties differ in the way they fill their heads, some having a large number of spikelets per head, others bearing a greater number of grains across the spikelet. There is thus ample scope for the plant breeder to attempt to build up a larger-sized head. Although climatic deficiencies demand a restricted population up to flowering (the time of maximum loss of moisture due to transpiration), it is possible that the average rainfall after that period would mature a greater number of grains than the present size of head carries.

The figures in the following table illustrate the variations in size of head.

Table V. *Showing distribution of sterility of spikelets in the ear and also number of grains per ear in good, average and poor heads under various circumstances.*

	Sterile spikelets at top	Spikelets bearing grain	Sterile spikelets at base	Grains per ear
Good Head (North-east, 1928) var. Nizam	0	13·8	4·5	17·5
Average (Wimmera, 1928) var. Federation	0·4	12·3	4·6	16·5
Poor Head (Central, 1928) var. Federation	2·4	8·6	3·6	11·6

With regard to size of grain, the figures are somewhat similar to those of the English crops. Under normal conditions in Victoria, there is sufficient rain in November to mature the grain to a good size. Varietal differences are a constant feature; Free Gallipoli in particular owes a great deal of its popularity to a very well filled grain.

THE MANURIAL PROBLEM.

Among the many developments of these census observations has been the study of the behaviour of wheat crops under various types of manuring. The main question of the application of superphosphate has given very interesting information.

Yield trials over the past 10 or 15 years have well demonstrated the benefit of applying dressings of superphosphate at seed time, and it is now regarded by farmers as a necessary accompaniment to their seeding operations. A phosphate deficiency has also been amply demonstrated by chemical analysis of these soils. The moot question at the present time is the optimum rate of application.

The figures from a harvest count on the Permanent Manurial Field at Werribee (Central district), during 1929, throw interesting light on the manner in which superphosphate affects the crops; and demonstrate in a very clear manner the importance of the information obtained from census observations. The plots in question have been cropped in alternate years since 1912, manure having been applied to the respective plots with the crop.

Table VI. *Harvest count on the permanent manurial field, Werribee, 1929.*

	No manure	Super-phosphate ½ cwt. per ac.	Super-phosphate 1½ cwt. per ac.	Super-phosphate 2 cwt. per ac.
Population: Plants per foot	5·45	8·13	7·72	7·75
Ears: Ears per plant	1·04	1·34	1·61	1·80
Ear size: Yield per ear (gm.)	0·40	0·84	0·86	0·88
Av. no. of grains per ear	11·6	18·5	20·1	20·9
No. of fertile spikelets per ear	7·9	10·7	11·0	11·8
Wt. of 1000 grains (gm.)	34·4	45·5	42·7	42·1
Yield: Bushels per acre	6·2	25·2	29·3	33·8

There are three main points of interest:

(1) There is a definite increase in the "p" factor for the manured plots. Counts made at various times have shown that this effect of superphosphate is not due to any actual increase in the germination of the seed, but rather to an increase in the vigour imparted to the young

plant, which enables it to get through the ground quicker, and later in life to withstand disease. This is most noticeable in those soils which tend to set. An important point is that after $\frac{1}{2}$ cwt. to the acre a further increase in the amount of superphosphate added causes no corresponding increase in "p."

(2) There is a definite rise in the production of heads per plant with increasing applications of superphosphate. This increase may act in a detrimental manner to the crop in a dry season, for it means greater vegetative activity, which will produce high yields in a good year, but may require too much moisture fully to develop in a dry year and so will result in what is called "burning-off." It is this factor which limits the most profitable rate of application of superphosphate in the drier northern areas.

(3) There is a further similar rise in size of head with increasing dressings of the manure. Grain size, however, apart from the first initial response, is governed more by the seasonal finishing conditions, and therefore does not show any further response to dressings of super-phosphate.

Thus, on this census information alone, it would be possible to predict that in the first case, it was essential that the superphosphate be applied with the seed (to give it the initial response), and secondly that the quantities of manure applied should be governed not only by the deficiency of the soil, but also by the available rainfall of the district concerned. Both these assumptions are actually borne out in practice.

The question of nitrogenous manuring, which is of such importance in England, has not received the same attention in Victoria. In the wheat-belt proper, top dressings of either ammonium sulphate or nitrate of soda have not shown any increased yields up to the present. Observations on the type of growth produced show that when nitrate is applied at seeding, spring growth is increased with a resultant raising of the tillering—that is, nitrates increase vegetative activity. It is apparent, however, that the type of growth produced is more "sappy," since the survival rate of tillers to heads is decreased. Since this type of growth is the exact opposite to that suited for Victorian conditions, the application of nitrate is not favoured. It is quite possible and indeed probable, that crops in certain areas of land in the colder portions of the State, which suffer from an excess of moisture during the winter months, will respond to a top dressing of nitrogenous manures.

ACCURACY OF RESULTS.

Experiments which have been conducted in Australia[8] have shown that the errors attaching to yield trials are of the same nature and order of magnitude as those determined in England. For this reason, the authors have followed the lead of the Cambridge School of Agriculture, and have adopted their methods of sampling.

There are two main points at issue:

(1) the accuracy with which the system of sampling obtains an estimate of the crop growth on the area studied;

(2) the justification of the comparison of varietal figures of tillering and so forth.

The former has been very exhaustively dealt with by Engledow in his earlier articles. The use of a "systematised" scatter of 100 "foot" samples has been found to be the most simple and practical method of attacking a census study. From time to time, the various figures have been tested for accuracy, and it has been found that for the population figure, the most variable of the four yield factors, ϵ_{12} (S.D. of difference of two means) is about 2 per cent., and in the other measurements, even greater accuracy is attained.

The practice in comparing varieties for census observation has been to study adjacent areas of $\frac{1}{4}$ to $\frac{1}{2}$ an acre in size, sown on special sites of similar land judged to be typical of the district. This method has proved quite satisfactory in indicating varietal differences in crop growth, since the big differences observed have proved to be constant in a given district.

It has been felt that some form of random replication of plots should be practised for the more accurate yield study of questions of manuring, etc., where the differences between treatments may be of smaller degree. Accordingly for these types of census study replicated plots are sown and suitable numbers of samples are drawn in a similar manner from the various plots.

SUMMARY.

Since the year 1927, observations have been made in Victoria on the development of wheat crops of various varieties grown in four different districts of the State.

The method used was based on that employed by Engledow in his "Investigations on Yield in Cereals," by taking counts on numbers of plants in various "foot length" samples scattered in a systematic manner over the areas studied.

The basis of the observations was the determination of the values

which comprise yield, viz. p = numbers of plants, e = ears per plant, n = number of grains per ear, g = average weight of grain.

In comparing the figures observed with those from the English crops, it was noted that the population factor, "p," showed important differences. Victorian conditions of climate in the wheat belt are such that it is essential that the rates of seeding and manuring shall be strictly limited, in order that the amount of early growth produced may be sustained to harvest under average conditions of rainfall.

Tillering and head production showed typical variations with spacing, manuring, soil and season. The values on the per plant basis actually showed little difference from the English figures, but this was undoubtedly affected by the smaller population.

Varietal studies of plant behaviour showed that the present tendency in plant breeding in Victoria was away from the original high-tillering sappy varieties to a conservative, lower-tillering and more sturdy type of growth better able to withstand drought conditions.

The values for the "percentage survival" of tillers to heads were a good indication of drought resistance. All the new wheats giving promise in yield trials showed an increase in this figure.

The third factor, "head size," showed an important decrease in number of grains per ear from the values for English crops, and illustrates the small head size produced in Victoria.

Average grain weights compare favourably with those from overseas.

Counts on plots receiving various quantities of superphosphate show that this manure increases the density of crop in both the factors of numbers of plants and the ear production per plant; it also increases size of head very materially.

Nitrogenous top dressing does not appear favourable for wheat in the general wheat belt, since it encourages a type of growth which is not drought resistant.

Every acknowledgment is made to Professor F. L. Engledow, the work reported being merely the results of the Victorian observations along the lines originated and developed by him at Cambridge.

Appendix.

As typical of the complete life history of various crops in Victoria, the following examples may be taken as illustrations. For uniformity of comparison, the first three crops shown are of the same variety, Free Gallipoli, now the most popular wheat grown in Victoria, and illustrate the way in which climatic conditions affect development. The fourth crop indicates the manner in which an extremely high possible yield was ruined by dry periods during the later portion of the year. A typical history of an English crop is also included.

Table VII. *Showing as specimens the data of the yield analyses of four Victorian wheat crops and one English crop for comparison.*

| | Good crop (North-east 1929) Free Gallipoli | Average (North-east 1928) Free Gallipoli | Poor crops | | English crop (Field L) Yeoman* |
			A (Central 1928) Free Gallipoli	B (Wimmera 1929) Wannon	
Population:					
Rate of seeding, lb. per ac.	73	88	89	75	150
No. of seeds sown per foot	10·6	12·9	12·5	14·0	—
Plants per foot at germination	8·67	10·3	6·32	12·7	19·7
Germination per cent.	82	80	51	91	—
Plants per foot at tillering	8·30	9·43	5·41	12·4	17·3
Plants per foot at harvest	8·15	8·56	3·87	8·06	17·5
Per cent. survival from germination	94	84	61	64	89
Tillers:					
No. of tillers per plant	4·30	3·14	2·90	4·93	4·7
No. of tillers per foot	35·8	27·2	15·7	61·1	81·4
Ears:					
No. of ears per plant	2·21	1·17	1·25	1·17	1·28
No. of ears per foot	18·0	10·0	4·84	9·45	22·4
"Percentage survival" per plant	51·4	37·2	42·0	20·6	27
"Percentage survival" per foot	50·5	36·8	30·8	15·5	27
Ear size:					
Yield per ear (gm.)	0·82	0·78	0·46	0·45	1·16
No. of grains per ear	17·8	18·4	12·6	12·2	27·1
Wt. of 1000 grains (gm.)	46·1	42·3	36·6	36·8	42·9
Yield:					
Bushels per acre	40·6	21·6	5·9	11·8	56·5

* Engledow (5).

In comparing the Free Gallipoli examples, the average crop (No. 2) failed to produce as vigorous a plant as the No. 1 crop, due primarily to the use of a poorer class of soil. The development of the plant (as shown by the tillering, 3·14 as compared with 4·30), does not show up to the same advantage; nor does the type of tiller produced reach the same high standard ("Percentage Survival" at heading of 37 per cent. compared with 51 per cent.). Subsequent head development does not show material differences.

The poor crop from the Central district (No. 3) shows the introduction of another factor. It failed for two reasons, in the first case, through low germination and plant survival, and secondly because of poor plant development. Both of these causes may be traced to the type of season experienced in the district. The crop was unfortunately sown rather late in soil which had not been worked deeply enough. Consequently, when the winter set in, cold and dry during June and July, not only did the seed germinate badly on account of the poor cover, but the young plants, with their roots in the cold surface soil, could not "over-winter," and where they did not die, developed but poorly. The survival to harvest was further affected by the appearance of a bad attack of "foot-rot" (*Fusarium* spp.), and a number of "white heads" were observed. The poor start of the plants during the winter months was shown in the low tillering and head production, and in addition, the ear development of those heads which matured grain, was also affected, until finally the yield was reduced to 6 bushels per acre.

The example of crop No. 4 is given to illustrate, firstly, the possible development in the black lands of the Wimmera, and secondly, the serious results which follow the production of bulky crops in dry seasons. Unfortunately for Wimmera farmers, the seed produced in the year prior to the case quoted was affected by a very dry period in November, when no rain was recorded. The sample for seeding during the following year was therefore pinched in spite of grading, and farmers, seeding at the usual rates, unconsciously sowed more grains per foot of drill row in the ground. A high germination (91 per cent.) resulted in a greater population per foot than usual, which further accentuated the bad conditions later in the year. Tillering was about normal and a very bulky crop promised a high yield. However, climatic conditions for the next three months, September, October and November, when only 2 in. of rain were recorded, dealt hardly with those tillers formed, and a survival to head production of only 21 per cent. was recorded. The dry period of late October and November greatly reduced size of head,

and the continued drought of early December further affected size of grain.

Had the season continued in a normal manner from August onwards and produced an average heading, a yield of approximately 40–50 bushels per acre would have been obtained. Actually the yield proved to be only 12 bushels per acre.

The English figures demonstrate the manner in which the higher yields are obtained from English crops.

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THE DIGESTIBILITY AND FEEDING VALUE OF DREG MEAL.

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THIS material which is also known as distillery dregs, dried dreg or slummage is a by-product of the production of alcohol. In this process a mixture of coarsely ground cereals is used and, after fermentation, the liquid extract is drawn off leaving behind the distillers' grains or draff.

A considerable amount of solid material is carried off in the extract and this later settles out and is known as distillers' dregs. The material is often used in a wet state but may be dried when it forms the highly concentrated foodstuff referred to as dreg meal.

COMPOSITION.

As might be expected, this material varies within fairly wide limits. The analysis of the sample investigated is given in Table I, together with analyses quoted in a leaflet published by the Department of Agriculture for Scotland[1].

Table I. *Composition of dreg meal (dried dregs).*

| | | Dreg meal | |
| | This experiment | Published results [1] | |
		%	Range
Moisture	5·5	10·0	7 –17
Crude protein	47·0	34·0	32 –40
Ether extract	13·9	14·0	9 –19
Nitrogen-free extractives	27·1	31·0	22 –31
Fibre	4·9	9·0	7 –11
Ash	1·6	2·0	2·1– 3·9

The ether extract and protein contents of dreg meal are high and the crude fibre is low. In the sample used in the experiments the crude protein was considerably higher (nearly 50 per cent.) than the average.

On its analysis this foodstuff would appear to have a very high feeding value. The ash content, however, is low; this is particularly the case with the calcium, of which it is almost devoid.

DIGESTIBILITY TRIAL.

A digestibility trial was carried out with sheep to obtain a true measure of the feeding value. Owing to the high oil and protein content, it was considered undesirable to feed the sheep a ration of dreg meal alone. Four sheep were taken which were known to be comparable and which had all given results of the same order in a previous trial on a sample of silage.

Two of the sheep were fed on a ration of artificially dried hay, the other two received a ration of the same hay and dreg meal.

The hay was of good quality, with a crude protein content of 14·55 per cent. dry matter.

The digestibility trial was carried out in the usual way in special metabolism boxes.

The sheep on hay alone received a ration of 900 gm. per head daily.

The sheep on dreg meal received 237·5 gm. of the meal and 700 gm. of hay per head daily.

Table II. *Digestibility coefficients of dreg meal (weights stated in gm.).*

Sheep A	Dry matter	Organic matter	Ether extract	Fibre	Nitrogen-free ex-tractives	Crude protein	True protein
Intake:							
Hay	4760	4365	112	1208	2353	693	599
Dreg meal	1796	1766	265	94	513	894	834
Total	6556	6131	377	1302	2866	1587	1433
Output in faeces:							
Total	1967	1707	62	356	747	517	494
From hay*	1350	1151	55	271	605	228	193
From dreg meal ...	617	556	7	85	142	289	301
Wt. of dreg meal digested	1179	1210	258	9	371	605	533
% of dreg meal digested	65·65	68·52	97·36	9·57	72·32	67·67	63·91
Sheep B							
Intake:							
Hay	4760	4365	112	1208	2353	693	599
Dreg meal	1559	1533	230	81	446	776	724
Total	6319	5898	342	1289	2799	1469	1323
Output in faeces:							
Total	1882	1623	61	322	751	522	441
From hay*	1350	1151	55	271	605	228	193
From dreg meal ...	532	472	6	51	146	294	248
Wt. of dreg meal digested	1027	1061	224	30	300	482	476
% of dreg meal digested	65·88	69·21	97·39	37·04	67·26	62·11	65·75
Average % of dreg meal digested	65·77	68·87	97·38	23·31	69·79	64·89	64·83

* Calculated from the weight of hay fed and the average digestibility coefficients of the hay as determined with the other pair of sheep.

After a pre-experimental period of five days the experimental period during which the excreta were collected was of eight days' duration.

The sheep did not eat the dreg meal readily at first, and a small quantity of treacle was added to it until they ate it freely. The total amount of treacle fed was 200 gm. and as this was fed partly during the pre-experimental period, no account was taken of it.

Any residue was removed from the mangers after each feed and weighed, the necessary subtraction being made from the day's feed.

With the exception of the value for the crude fibre there is a good agreement between the sheep. The fibre would appear to be of low digestibility but, since the amount present in the dreg meal is low, the variation in the values obtained will not affect the calculation of the nutrients.

FEEDING VALUE.

The digestible constituents of the dreg meal are given in Table III along with other concentrated foods which are comparable.

Table III. *Digestible constituents of dreg meal and comparable foods.*

	Dreg meal		Linseed cake	Sesame cake	Sunflower cake
	This experiment %	Published values [1] %	%	%	%
Moisture in foodstuffs ...	5·5	10·0	11·0	9·5	9·2
Crude protein	30·50	25·5	28·8	35·8	35·5
Ether extract	13·57	12·1	7·9	11·3	11·1
Nitrogen-free extractives ...	18·89	19·0	25·4	11·5	14·7
Crude fibre	1·15	5·6	4·3	2·1	3·5
True protein	28·43	*	27·2	34·2	32·4
Starch equivalent per 100 lb.	72·68	*	71·8	71·0	72·0

* Not quoted.

The starch equivalent has been calculated by the method of Kellner[2], the correction for fibre being made by deducting 0·58 per cent. of starch equivalent for each 1 per cent. of crude fibre.

Dreg meal is similar in feeding value to linseed cake and to the lesser known sesame and sunflower cakes. The figures in column 2 are quoted in the leaflet mentioned above, and are similar except that they are somewhat lower in crude protein than the sample under investigation.

Dreg meal is thus a valuable foodstuff of high digestibility and feeding value, which may replace linseed cake. Owing to its high oil content, however, it should be used with caution and cattle should not

receive a greater allowance than 4–5 lb. per head per day. Owing to its low fibre content, it should be a suitable foodstuff for pigs as well as cattle and when the price is favourable would form a useful source of protein in the ration.

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INVESTIGATIONS INTO THE INTENSIVE SYSTEM OF GRASSLAND MANAGEMENT.

BY THE AGRICULTURAL RESEARCH STAFF OF IMPERIAL CHEMICAL INDUSTRIES, LIMITED.

IV. THE DIGESTIBILITY AND FEEDING VALUE OF ARTIFICIALLY DRIED GRASS.

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(With One Text-figure.)

INTRODUCTION.

GRASSLAND farmers have two alternatives from which they may choose when deciding how to preserve their surplus grass. They may make hay or ensilage. Researches carried out at Jealott's Hill, of which the present paper is an instalment, have for their object the preservation of grass by drying and making it into cake or meal. The full account of these researches will be published elsewhere. The object of the present contribution is to describe the findings obtained as to the digestibility and feeding value of dried grass. A later paper in this series will describe the effect of different methods on the digestibility of the material as compared with that of the original grass.

Material and method of drying.

The grass used was obtained from an area which had been intensively treated and which had received a dressing of nitrogen four weeks prior to the date of cutting. The grass was dried in an experimental band drier erected at Jealott's Hill. The grass is carried on a perforated travelling band in a thin layer through a series of compartments in which heated air is circulated. The air has an inlet temperature of 200° C. and is drawn through a coke furnace. The moisture content is reduced to approximately 5 per cent. in the course of about 20 minutes and the material retains its green colour. The dried grass is then either compressed into bales or briquettes or may be ground into the form of a meal. Two samples of

the material dried in this plant have been examined for digestibility. One sample had been ground and was fed in a meal form, the other was used without further treatment after the drying process.

COMPOSITION.

The composition of these two samples is given in Table I.

Table I. *Composition of artificially dried grass.*

	Dried grass		Grass meal	
	% of original	% of dry matter	% of original	% of dry matter
Moisture	12·00	—	15·50	—
Ether extract	4·76	5·41	3·09	3·66
Fibre	16·17	18·38	17·12	20·26
Crude protein	19·10	21·70	15·08	17·85
Ash	9·96	11·32	10·83	12·82
Nitrogen-free extractives	38·01	43·19	38·38	45·41
	100·00	100·00	100·00	100·00
Organic matter	78·04	88·68	73·67	87·18
True protein	17·40	19·77	13·70	16·21
Calcium oxide (CaO)	0·97	1·11	0·68	0·80
Phosphoric acid (P_2O_5)	0·77	0·88	0·59	0·70
Potash (K_2O)	2·82	3·20	*	*
Ratio true : crude protein	0·91	0·91	0·91	0·91

* Not determined.

The grass for both samples was cut during the end of August and beginning of September, 1929. The grazing season of 1929 was marked at Jealott's Hill by a prolonged drought which restricted growth during the months of June and July and it was only after rain fell in August that it was possible to cut some short leafy grass for drying.

Although the figures for crude protein are fairly high and much superior to those which are obtained in hay, they are not so high as those generally obtaining in grass under intensive treatment, as quoted by Greenhill[1].

The grass grew rapidly in the early autumn but, owing to contamination with stemmy material resulting from the dry summer period, was lower in crude protein content and higher in crude fibre than might be expected.

DIGESTIBILITY TRIALS.

Four Suffolk cross wether sheep were used in the trials, one of which was carried out in September, 1929, the other in March, 1930. The animals were confined in metabolism crates and the faeces and urine were collected separately by means of metabolism harness of the usual type.

The experimental period in the case of the grass meal was only of eight days' duration, but with the dried grass an experimental period of ten days was possible. In both trials a preliminary period of four days was allowed before the experiment commenced.

Digestibility of the dried grass.

The digestibility coefficients of these samples have been calculated in the usual way and are given in Table II.

Table II. *Digestibility coefficients of dried grass (per cent. digested).*

	Organic matter	Ether extract	Crude fibre	Nitrogen-free ex-tractives	Crude protein	True protein
Sheep C	77·20	60·07	80·46	77·58	77·08	77·86
Sheep D	75·52	59·15	77·84	76·22	75·61	75·12
Average: C and D	76·36	59·61	79·15	76·90	76·35	76·49

The agreement between the sheep is very satisfactory and the digestibility coefficients generally are of a high order. The digestibility of the crude fibre is outstanding and shows that this constituent is of considerable feeding value.

Nitrogen balance.

A balance sheet showing the utilization of the nitrogen is given in Table III. It shows that a definite retention of nitrogen has taken place and as would, therefore, be expected an increase in the weight of the sheep occurred. Sheep C and D gained respectively 1 lb. 6 oz. and 1 lb. 0 oz.

Table III. *Nitrogen balance.*

	gm.
Mean daily intake	36·4
Voided daily in faeces and urine	32·4
Mean daily balance	+4·0

Mineral balance.

A study of the balance of the ash constituents shows that the grass is capable of supplying the needs of the sheep so far as the calcium, phosphoric acid and potash are concerned and that the animals have not been called upon to sacrifice any of these constituents from their bodies. In fact the sheep have been able to store considerable amounts of potash and to a less marked extent phosphoric acid.

Table IV. *Balance sheet showing distribution of calcium,*
phosphorus and potassium (stated in gm.).

	CaO	P_2O_5	K_2O
Mean daily intake	11·59	9·21	33·55
Voided daily in faeces and urine	11·56	8·38	31·05
Mean daily balance	+0·03	+0·83	+2·50

Digestibility of grass meal.

The digestibility coefficients calculated from the analytical data are given in Table V. It will be seen that the agreement between the two sheep is very good and the remarks on the digestibility of the dried grass apply equally well to the grass meal. The sheep ate the grass meal readily and drank a notable quantity of distilled water. For several days in the pre-experimental period the meal was fed in the dry state and it was found that the sheep blew it about and so lost a portion of their ration. To remedy this the meal was mixed with distilled water; in this state it was relished by the sheep and no loss occurred.

Table V. *Digestibility coefficients of grass meal (per cent. digested).*

	Organic matter	Ether extract	Crude fibre	Nitrogen-free extractives	Crude protein	True protein
Sheep C 1	78·74	72·61	80·41	80·17	75·63	75·22
Sheep D 1	78·37	72·61	79·31	79·61	75·45	74·43
Average: C 1 and D 1	78·56	72·61	79·86	79·89	75·54	74·83

Nitrogen balance.

Here again the balance sheet shows a retention of nitrogen in the case of both sheep. The weights of the sheep before and after the trial showed a gain in live weight (1 lb. 4 oz. for sheep C 1, and 8 lb. 0 oz. for sheep D 1), which is in accord with the findings of the nitrogen balance.

Table VI. *Nitrogen balance.*

	gm.
Mean daily intake	22·4
Voided daily in faeces and urine	17·5
Mean daily balance	+4·9

The mean daily balance was +5·2 gm. for Sheep C 1 and +4·8 gm. for Sheep D 1.

Mineral balance.

The mineral balance is set out in Table VII. In the case of the grass meal potash was not determined. The figures show that the calcium was in a state of equilibrium and there was a retention of phosphoric acid.

Table VII. *Balance sheet showing the distribution of calcium and phosphorus (stated in gm.).*

	CaO	P_2O_5
Mean daily intake	6·3	5·5
Voided daily in faeces and urine	6·4	4·8
Mean daily balance	−0·1	+0·7

FEEDING VALUE.

Woodman *et al.* (2) have recently published the results of a digestibility trial carried out on grass dried artificially. The grass was cut at weekly intervals and dried in steam-heated troughs, the herbage being mechanically agitated to facilitate the drying. The dried product was later compressed into cakes by means of a hydraulic press. The grass was of excellent quality containing 26 per cent. crude protein and only 15·4 per cent. crude fibre. In his paper, Woodman compares the digestibility of the artificially dried grass with that of herbage cut at weekly intervals and fed in the fresh condition. These figures are reproduced here for comparison with the dried grass and grass meal under investigation.

Table VIII. *Digestibility coefficients of dried grass and weekly-cut herbage fed in the fresh condition.*

	Dried grass % (Jealott's Hill)	Grass meal % (Jealott's Hill)	Dried grass cake % (Woodman)	Fresh grass average 1925 season % (Woodman)	Fresh grass average 1926 season % (Woodman)
Organic matter	76·4	78·6	78·1	78·7	79·9
Crude protein	76·4	75·5	78·2	80·6	82·4
Ether extract	59·6	72·6	70·6	54·1	60·3
Nitrogen-free extractives	76·9	79·9	79·3	80·5	80·1
Crude fibre	79·1	79·9	78·3	78·5	81·9

The figures relating to the fresh grass are the average values throughout the season. The comparison shows that the digestibility of the crude protein of the dried grass and grass meal was slightly lower than the values quoted by Woodman for the dried grass cake and fresh grass. In view of the contamination of the grass with a small amount of old growth, mentioned above, this is to be expected. On the whole, the values for the grass meal and for the dried grass are of the same order, showing that the material had a high digestibility. A satisfactory feature of the figures is the high value obtained for the crude fibre. This puts the dried grass and grass meal in the class of concentrated foodstuffs and proves both decidedly superior to hay or similar roughages.

In Table IX the digestible nutrients of the dried grass and grass meal are compared with those of fresh grass (calculated on a basis of 12 per cent. moisture), excellent and good meadow hay.

Table IX. *Digestible nutrients in dried grass, fresh grass, excellent and good meadow hay.*

	Dried grass %	Grass meal %	Fresh grass (3) %	Excellent meadow hay (4) %	Good meadow hay (4) %
Moisture	12	15	12	16	15
Digestible crude protein	14·58	11·46	14·66	9·2	5·4
Digestible ether extract	3·31	2·26	2·97	1·5	1·0
Digestible nitrogen-free ex-tractives	29·23	30·84	34·01	30·1	25·7
Digestible fibre	12·80	13·75	12·00	12·7	15·0
Digestible true protein	13·31	10·31	*	6·5	3·8
Starch equivalent per 100 lb.	51·48	48·61	61·06	40·6	31·0
Nutritive ratio	1 : 3·44	1 : 4·38	1 : 3·63	1 : 5·05	1 : 7·99

* Not quoted.

The figures for the fresh grass are calculated from those quoted in a paper by Woodman *et al.* for grass cut at three-weekly intervals in 1928 (3), whilst the figures for the hays are given by Kellner (4).

The high digestible crude protein content and the narrow nutritive ratio of the dried grass are very similar to those of the fresh grass and are very different from the values for the hays. The values for the grass meal show that this material is of much greater value than the hay although not quite equal to the fresh grass. It should be borne in mind, however, that the composition of the grass meal was not what one would expect under normal conditions when a crude protein content of 20 to 25 per cent. should be obtainable.

The chief point of interest is that material of a high order of digestibility is obtainable as a result of artificial drying.

The two samples under investigation are balanced for milk production. The standard which may be accepted for this purpose is 2·25 to 2·5 lb. of starch equivalent containing 0·5 to 0·6 lb. of digestible true protein per gallon of milk produced. The following rations would cater for this:

(i) 4¼ lb. of the dried grass described above containing 2·22 lb. of starch equivalent including 0·55 lb. of digestible protein.

(ii) 5¼ lb. of the grass meal described above containing 2·55 lb. of starch equivalent including 0·55 lb. of digestible protein.

The former would be more satisfactory on account of the smaller amount it would be necessary to feed per gallon of milk.

FEEDING TRIAL WITH DAIRY COWS.

A group of five cows was selected from the herd and a feeding trial was arranged to test the feeding value of artificially dried grass, of which a supply of 5 cwt. was available. The analysis varied from 18 per cent. to 21 per cent. of crude protein in the dry matter. It was decided to
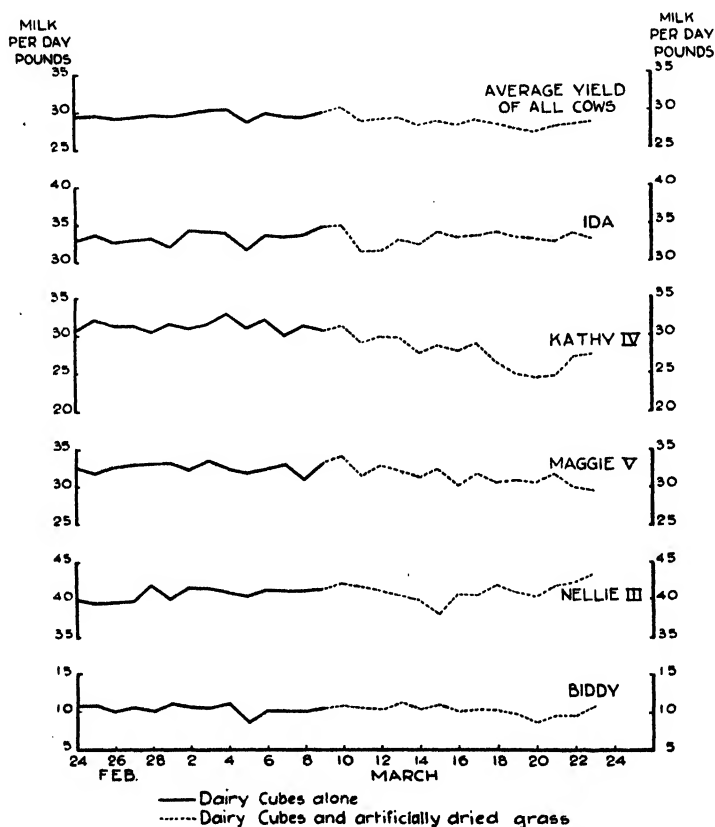
Fig. 1.

replace a balanced dairy ration by the dried grass on a basis of equal weights.

The five cows selected had been receiving a standard maintenance ration and were fed a compound dairy cube containing 71 per cent. of starch equivalent including 16 per cent. of digestible protein at the rate of 3½ lb. for every gallon of milk produced.

The maintenance ration of 40 lb. mangolds and 15 lb. hay per head was continued and a basal ration of:

> 40 lb. mangolds,
> 15 lb. hay,
> $5\frac{1}{4}$ lb. balanced dairy cubes,

was fed to four of the cows. An allowance of $3\frac{1}{2}$ lb. of dried grass per gallon was fed for every gallon produced over $1\frac{1}{2}$. The four cows were producing respectively $3\frac{1}{2}$, 3, 3 and $4\frac{1}{4}$ gallons of milk per day and received 7, $5\frac{1}{4}$, $5\frac{1}{4}$ and $10\frac{1}{2}$ lb. of dried grass in addition to the basal ration.

The fifth cow (Biddy) which had been receiving 4 lb. hay, 15 lb. of straw and $3\frac{1}{2}$ lb. of balanced dairy cubes was fed the same amounts of hay and straw but the cubes were replaced by $3\frac{1}{2}$ lb. of dried grass. The dried grass ration was fed from March 10 to March 23 inclusive, a period of 14 days. The total yields during this period and the preceding fortnight are given in Table X. The results are very satisfactory since no animal has shown a marked diminution in yield. That some diminution was to be expected is evident from the fact that at least $4\frac{1}{4}$ lb. were necessary to produce a gallon of milk, whereas in this trial only $3\frac{1}{2}$ lb. were fed per gallon.

Table X. *Total milk yield of cows (in lb.).*

	Period 1 Dairy cubes fed	Period 2 Dried grass fed
Ida	$466\frac{1}{4}$	460
Kathy IV	$435\frac{3}{4}$	$382\frac{1}{2}$
Maggie V	452	429
Nellie III	$569\frac{3}{4}$	$570\frac{1}{4}$
Biddy	144	$142\frac{3}{4}$
Total	$2,067\frac{3}{4}$	$1,984\frac{1}{2}$

Kathy and Maggie are responsible for the greater part of the decrease. The daily yields have been plotted in graphical form (Fig. 1) and it will be noticed that the effect on the average yield of the individual cows is but small in all cases except that of Kathy. This cow was, however, picking up again towards the end of the period. Unfortunately the supply of dried grass was not sufficient for a more protracted trial, but the indications are that artificially dried grass is readily eaten by dairy cows and will produce the quantity of milk for which the nutrients it supplies are theoretically capable.

SUMMARY.

Digestibility trials have been carried out with sheep on a sample of dried grass and a grass meal, both of which were artificially dried on an experimental band drier. The analyses of the two materials were affected by the fact that the grass was cut in August after a very dry summer and the herbage was contaminated with a certain amount of stemmy material.

The digestibility of the two samples is of a high order and, with the exception of that of the crude protein, equal to the values quoted by Woodman for short grass. The sheep put on weight during both trials and there was a retention of nitrogen and mineral matter in all cases. The dried grass would appear to contain an adequate amount of available calcium, phosphorus and potash for the plane of nutrition at which it was fed.

The high digestible crude protein content and narrow nutritive ratio of the dried grass and grass meal prove the material to be superior to excellent hay despite the fact that both samples had a lower crude protein content than would normally be obtained under conditions of intensive treatment.

A feeding trial with dairy cows in which the major part of the production ration was replaced by the dried grass has shown that artificially dried grass was readily eaten by dairy cows and would produce the quantity of milk for which the nutrients it supplied were theoretically capable.

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APPENDIX I.

Digestibility coefficients of dried grass.

	Organic matter gm.	Ether extract gm.	Crude fibre gm.	Nitrogen-free ex-tractives gm.	Crude protein gm.	True protein gm.
Sheep C		Dried grass eaten 11,996 gm.				
In dried grass eaten	9,361	571	1,940	4,559	2,291	2,087
In faeces	2,134	228	379	1,022	525	462
Weight digested	7,227	343	1,561	3,537	1,766	1,625
Per cent. digested	77·20	60·07	80·46	77·58	77·08	77·86
Sheep D		Dried grass eaten 11,829 gm.				
In dried grass eaten	9,232	563	1,913	4,496	2,259	2,058
In faeces	2,260	230	424	1,069	551	512
Weight digested	6,972	333	1,489	3,427	1,708	1,546
Per cent. digested	75·52	59·15	77·84	76·22	75·61	75·12
Average digested, %	76·36	59·61	79·15	76·90	76·35	76·49

APPENDIX II.

Weights of sheep.

	Sheep C		Sheep D	
	lb.	oz.	lb.	oz.
Before trial	140	10	129	0
End of trial	142	0	130	0
Gain	1	6	1	0

APPENDIX III.

Digestibility coefficients of grass meal.

	Organic matter gm.	Ether extract gm.	Crude fibre gm.	Nitrogen-free ex-tractives gm.	Crude protein gm.	True protein gm.
Sheep C 1		Grass meal eaten 7,425 gm.				
In grass meal eaten	5,471	230	1,271	2,849	1,120	1,017
In faeces	1,163	63	249	565	273	252
Weight digested	4,308	167	1,022	2,284	847	765
Per cent. digested	78·74	72·61	80·41	80·17	75·63	75·22
Sheep D 1		Grass meal eaten 7,425 gm.				
In grass meal eaten	5,471	230	1,271	2,849	1,120	1,017
In faeces	1,183	63	263	581	275	260
Weight digested	4,288	167	1,008	2,268	845	757
Per cent. digested	78·37	72·61	79·31	79·61	75·45	74·43
Average digested, %	78·56	72·61	79·86	79·89	75·54	74·83

APPENDIX IV.

Weights of sheep.

	Sheep C 1		Sheep D 1	
	lb.	oz.	lb.	oz.
Before trial	77	0	81	0
End of trial	78	4	89	0
Gain	1	4	8	0

APPENDIX V.

Daily and total milk yields of cows.

Period 1	Ida	Kathy IV	Maggie V	Nellie III	Biddy	Total
			Dairy cubes fed			
February 24	33	30¾	32½	40	10¾	147
,,　　25	33¾	32	31¾	39½	10¾	147½
,,　　26	32¼	31¼	32½	39¾	10	146¼
,,　　27	33	31¼	32¾	39¾	10½	147¼
,,　　28	33¼	30¼	33	42	10	148¼
March 1	32	31½	33	40	11	147½
,,　　2	34½	30¾	32	41½	10½	149¼
,,　　3	34	31¼	33¼	41½	10½	150¾
,,　　4	33¾	32¾	32	41	11	150¼
,,　　5	31½	30¾	31½	40¼	8½	142¼
,,　　6	33¼	32	32	41¼	10	148¾
,,　　7	33¼	29¾	32½	41	10	146½
,,　　8	33½	31	30½	41	10	146
,,　　9	34½	30¼	32¾	41¼	10½	149¼
Total	466¾	435¼	452	569¾	144	2067¾

Period 2	Ida	Kathy IV	Maggie V	Nellie III	Biddy	Total
			Dried grass fed			
March 10	34¾	31	33½	42	10¾	152
,,　　11	31¼	28¾	31	41½	10½	143
,,　　12	31¼	29½	32½	41	10¼	144¼
,,　　13	32¼	29¼	31¼	40¼	11¼	145
,,　　14	32	27¼	30¼	39¾	10¼	140
,,　　15	33¾	28¼	31¾	37¾	11	142½
,,　　16	33	27½	29½	40¼	10	140¼
,,　　17	33¼	28½	31	40¼	10¼	143¼
,,　　18	33¾	26	29¾	41½	10¼	141¼
,,　　19	33	24½	30	40½	9¾	137¾
,,　　20	32¾	24	29¾	40	8¾	135¼
,,　　21	32¼	24¼	30¾	41¼	9¼	138
,,　　22	33½	26¾	29	41¾	9¼	140½
,,　　23	32¾	27	28½	42¾	10½	141¼
Total	460	382½	429	570¼	142¾	1984¼

INVESTIGATIONS INTO THE INTENSIVE SYSTEM OF GRASSLAND MANAGEMENT.

By the Agricultural Research Staff of Imperial Chemical Industries, Limited.

V. THE DIGESTIBILITY AND FEEDING VALUE OF GRASS SILAGE MADE IN A TOWER, AND THE DIGESTIBILITY AND COMPARATIVE YIELD OF ARTIFICIALLY DRIED GRASS OBTAINED FROM THE SAME SOURCE.

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(With Two Text-figures.)

In May, 1929, it was decided to make some grass silage using a circular steel silo. The silo was only 8 feet high and a drain was fitted in the centre connecting with the outside.

The drain was constructed of 6 inch glazed pipe and shaped as in Fig. 1, the concrete floor of the silo being given a slight slope down to the outlet which was covered with a square tile with fine perforations. In order to prevent the silage coming into direct contact and blocking the fine holes some rough concrete blocks were built round the tile to keep the silage off it.

The grass used was obtained from a field of permanent grass which had reached a stage when it might have been cut for early hay and when some of the earlier grasses were showing a "head." There was not much clover in the herbage.

Cutting was started on the morning of May 27 and filling was commenced at 1 p.m. on that date. When two loads had been put in the silo, a sack of grass was embedded in the centre. The sack was of open-mesh jute protected by enveloping it in a fine wire mesh cage. A sub-sample of the grass was taken at the same time, the contents of the sack being weighed.

Two maximum thermometers enclosed in iron cylinders were also placed in the silage, one on either side of the sack.

A further load of grass was put on the top that evening. The first

sack in the silo was originally at a height of 4 ft. from the bottom of the silo.

On the morning of the 28th a second sack accompanied by two maximum thermometers was placed in the silo at a height of 7 ft. from the bottom. The silo was then completely filled and built up to a height of 5 ft. above the level of the sides.

In filling the silo the centre was kept well up, the sides being trodden. The object of this was to make the silage settle down towards the wall of the silo and to prevent any shrinkage away from the walls later on.
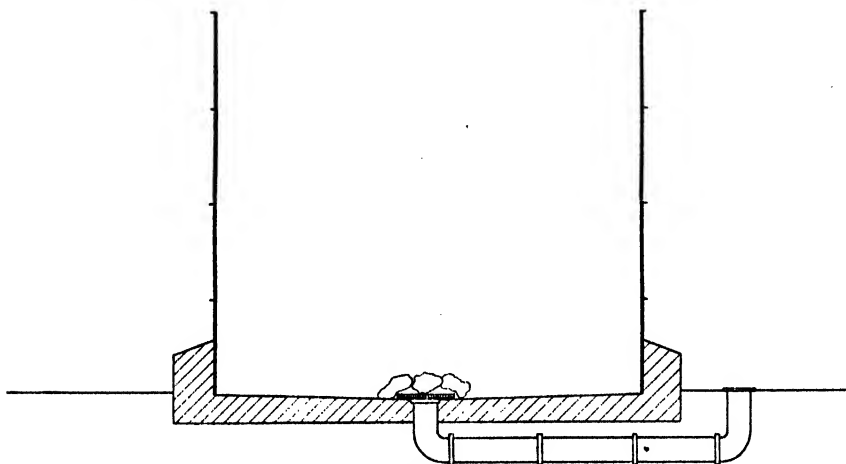
Fig. 1.

On May 29 the silage had settled slightly, no drainage being apparent, and the two doors of the silo which had been luted with bitumen showed no leakage.

On May 31 the silage had settled to the level of the top of the silo, there was a very small amount of effluent and no signs of leakage round the sides.

A load of grass from another field was filled in on June 2 and a further load on June 3, by which date there was still no sign of any amount of effluent. Further loads were put on on June 4, 6 and 7 to a height of 5 ft. above the sides. This had settled to the top of the silo by June 10 and on the 15th the silo was finally built up to a height of 6 ft. above the sides with waste grass cut from odd areas. This material was put on primarily to act as a protective cover and settled to the top of the silo, leaving a dome-shaped top to shed water. No further covering was put on the silo, which was exposed to the weather.

EFFLUENT.

The first lot of effluent was collected on June 21 when the drain was full. The effluent was pumped away and it was found that the drain when full held exactly 16 litres. Whenever it was completely filled the drain was emptied. The effluent was sampled every time and this sample was analysed. The amount collected up to January 8, 1930, was 624 litres.

No further collection was made after this date as the drain did not fill up again and the analytical figures showed that the losses were small.

The amount of effluent coming away diminished after August but increased again considerably in December on account of heavy rainfall. After the middle of January little rain fell and the amount of effluent was negligible. The loss in November and December would have been obviated if some impervious covering had existed.

The samples taken from the effluent were kept under a layer of toluene and were analysed as soon as possible. The dry-matter content of the effluent was determined by methods similar to those used for the estimation of total solids in milk. The amount of ash in this dry matter was estimated. The total nitrogen was determined by the Kjeldahl method, the amino nitrogen in the apparatus devised by Van Slyke[1]. Ammoniacal nitrogen was determined by distillation with magnesium oxide. The volatile acids and acidity of the alcohol extract were determined by Woodman's modification of Foreman's method[2].

The full analytical results are given in the appendix and show some very interesting features. Samples 1, 2 and 3 were mixed and analysed as one. All the other samples, 4–15, were composite samples of three consecutive collections of effluent.

The amount of dry matter per unit volume increases up to the end of November and then drops markedly, a statement which is also true of the ash in the effluent, though in the latter case the maximum is reached in mid-September. The nitrogen shows the same general trend, the amount in the effluent being at its maximum at the end of September. The ammoniacal and amino nitrogen show an increase up to mid-November, falling off subsequently.

The volatile acids do not show any great differences, though there is a marked increase in the amount in the fourth sample as compared with the composite of the previous three. The volatile acids in the last three samples show a gradual diminution, but prior to this there is no sign of a progressive formation of these acids. It would appear that the formation of volatile acids takes place in the early stages. This view is also

upheld by the acidity of the alcohol extract of the effluent which shows a marked increase in acidity in the fourth sample as compared with the one preceding it, but is a variable figure, thereafter, gradually decreasing in the last five samples. The increase in the undetermined nitrogen after the first sample is evidence of the decomposition of the nitrogenous substances as a result of fermentative changes, reaching a maximum by the end of August.

The total loss of dry matter in the drainage was 81 lb., which represents about 1·5 per cent. of the dry matter of the whole of the edible silage, which was computed at 10 tons. The ash formed a high proportion of the dry matter in the drainage, but assuming that the whole of the silage contained 10 per cent. of ash, calculated on a dry-matter basis, this only represents a loss of 4 per cent. of the total ash present.

The loss of nitrogen, calculated in the same manner, only amounts to 3·5 per cent. of the total nitrogen present, not a serious loss. The majority of the nitrogen in the effluent was in the ammoniacal or amino form, the proportion of undetermined nitrogen being 15 per cent.

It is clear that the losses in the drainage are but small, and from recent work in which the drain was closed it would seem that this loss can be obviated entirely since there was no leakage at all in the latter case.

LOSSES DURING THE FERMENTATION PROCESSES.

The sacks embedded in the silo were removed as they were reached and the sacks and their contents were weighed. It was found that there was very little material adhering to the outsides of the sacks themselves as the wire-mesh cages had prevented the silage being pressed directly against the sacking. The weights of the sacks are given in Table I. The

Table I. *Weights of grass and silage in the sacks embedded in the tower silo.*

			lb.	oz.
1. Lower sack (No. 1)		Weight of sack plus grass	25	8
27. v. 29		Weight of sack	1	4
		Weight of grass	24	4
24. iii. 30		Weight of sack plus silage	22	0
		Weight of sack	1	4
		Weight of silage	20	12
2. Upper sack (No. 2)		Weight of sack plus grass	38	11
28. v. 29		Weight of sack	1	7
		Weight of grass	37	4
7. iii. 30		Weight of sack plus silage	35	6
		Weight of sack	1	7
		Weight of silage	33	15

lower sack was obtained at a height of 1 ft. from the bottom of the silo, the upper sack at 1 ft. 6 in. above the lower.

This represents a loss of 14·4 and 8·9 per cent. for the lower and upper layers respectively, of the fresh weight of grass ensiled.

The analytical figures for the original grass and the silage made from it are given in Table II together with average values for both sacks. A consideration of the average values shows an increase in all constituents except the nitrogen-free extractives and true protein which are markedly lower in the case of the silage. In the mineral matter the percentage of calcium increases, whereas that of the phosphorus has decreased.

Table II. *The composition of the original grass and the silage made therefrom.*

	Sack No. 1 Lower layer		Sack No. 2 Upper layer		Average Sacks 1 and 2	
	Grass 27. v. 29	Silage 24. iii. 30	Grass 28. v. 29	Silage 7. iii. 30	Grass	Silage
Moisture in fresh material ...	75·94 %	73·45 %	73·85 %	76·00 %	74·9 %	74·73 %
	% dry matter	% dry matter	% dry matter	% dry matter	% dry matter	% dry matter
Ether extract	3·64	4·17	3·28	4·49	3·46	4·33
Fibre	23·58	27·54	24·41	29·01	23·99	28·28
Crude protein	14·06	15·09	13·81	14·47	13·93	14·78
Ash	7·75	10·10	7·96	9·70	7·86	9·90
Nitrogen-free extractives ...	50·97	43·10	50·54	42·33	50·76	42·71
	100·00	100·00	100·00	100·00	100·00	100·00
Organic matter	92·25	89·90	92·04	90·30	92·15	90·10
True protein	12·20	7·55	11·50	8·09	11·85	7·82
Lime (CaO)	0·64	0·80	0·58	0·74	0·61	0·77
Phosphoric acid (P_2O_5) ...	0·72	0·56	0·73	0·70	0·73	0·63
Ratio true : crude protein ...	0·87	0·50	0·83	0·56	0·85	0·53

The most noticeable feature of all is the decrease in the proportion of true protein; this has decreased from 85 to 53, a diminution of 36·1 per cent.

The actual weights of the constituents have been calculated from the data for the weights of the sacks and the analysis of their constituents before and after the process of ensiling. The figures can only be approximate in view of the possible magnitude of sampling errors.

The figures show that there has been a definite loss of phosphorus and nitrogen-free extractives and a very marked loss in true protein. The figures for the ash seem to show that a good deal of leaching has occurred, the lower layer having increased in total ash and lime, whereas the upper layer does not appear to have altered greatly in respect of

these constituents. None of the other constituents shows much change, since it is unlikely that a difference of less than 10 to 15 per cent. would be large enough to eliminate the possibility of experimental error on two samples. It must be remembered also that the silage under consideration only filled half of the silo and that no data are available for the super-imposed material from which some of the soluble material must have passed down into the lower half of the silo.

Table III. *Gain or loss of constituents present in the original grass as compared with the silage (stated as percentages).*

	Sack No. 1 Lower layer	Sack No. 2 Upper layer
Ether extract	+ 8·0	+14·5
Fibre	+10·0	+ 0·0
Crude protein	+ 1·5	− 12·5
Ash	+23·0	+ 2·0
Nitrogen-free extractives	−20·0	−30·0
Dry matter	− 6·0	−16·5
Organic matter... ...	− 8·0	−18·0
True protein	− 41·5	− 41·0
Lime (CaO)	+16·5	+ 7·0
Phosphoric acid (P_2O_5)	−27·0	−20·0

TEMPERATURE.

The temperatures registered were comparatively low, the readings close to the lower sack being 101° F. and 100·5° F., whilst in the vicinity of the upper sack 103° F. and 104° F. were registered.

DIGESTIBILITY.

Two one-year old Kerry cross wethers were used for the digestibility trial and were confined in metabolism boxes, the urine and faeces being collected separately by means of harness of the usual type. The silage for the trial was cut out in a block from the material lying between the two sacks embedded in the silo. A fresh surface was exposed every day and a feed of 3630 gm. per sheep was weighed out, an aliquot sample being taken at the same time for dry-matter determination. The dry-matter content did not vary much and the average over the whole period was 24·2 per cent. The daily feed was fed in two approximately equal portions. Any residue was collected, dried and calculated in terms of fresh silage.

The sheep cleaned up their feeds very well and there were uneaten residues only on two occasions.

The average analysis of the two sacks was taken as the composition of the silage fed.

The silage was of a light brown colour, with a pleasant aroma and there was no evidence of objectionable acidity. In the analysis the volatile substances, which would be lost during the drying of the samples, have not been taken into account, since it is generally found that they will not greatly affect the results. The analysis of the silage is given in Table II. The digestibility coefficients were calculated in the usual way.

Table IV. *Digestibility coefficients of grass silage.*

	Sheep A	Sheep B	Mean
Dry matter	71·57	71·91	71·74
Organic matter	72·88	73·16	73·02
Ether extract	76·69	78·67	77·68
Fibre	76·24	78·27	77·26
Nitrogen-free extractives	71·86	71·59	71·73
Crude protein	67·92	67·48	67·70
True protein	50·75	48·34	49·55

The agreement between the two sheep is very satisfactory and the values for the digestibility are high for grass silage as may be seen from Table V.

Table V. *Digestibility coefficients of different types of silage (stated as percentages).*

	Grass silage made in a tower at Jealott's Hill	Rye grass and clover silage (3) made in a stack	Grass silage (4) from heap*	Grass silage (4) from pit*
Dry matter	71·7	47·2	—	—
Organic matter	73·0	49·3	—	—
Crude protein	67·7	12·2	50	70
Ether extract	77·7	70·0	48	50
Nitrogen-free extractives	71·7	55·9	58	58
Crude fibre	77·3	53·8	60	58

* Calculated from the data given for total and digestible constituents.

The digestibility of the crude protein in the grass silage made in a pit, quoted by Kellner (4), is the only instance in which the values for the grass silage made in the tower are equalled and, generally speaking, it is superior in all respects. It is only fair to say that the case quoted by Woodman (3) is an abnormal one.

The figures in Table VI give the amounts of digestible constituents in the tower silage, and for comparative purposes the values given by Kellner for the digestible constituents of silage made in a heap and in a pit are also included together with those for average meadow hay.

Table VI. *Digestible nutrients in the silage made from grass in a tower, heap and pit, and meadow hay (stated as percentage of original material).*

	Tower grass silage	Grass silage (4) from pit	Grass silage (4) from heap	Average (4) meadow hay
Moisture in original material ...	75·8	80·6	68·0	14·3
Digestible crude protein	2·4	1·4	1·9	5·4
Digestible ether extract	0·8	0·4	1·3	1·0
Digestible nitrogen-free extractives	7·4	4·7	7·5	25·7
Digestible crude fibre	5·3	3·8	5·9	15·0
Digestible true protein	0·9	0·9	0·7	3·8
Starch equivalent per 100 lb. ...	12·6	7·9	12·3	31·0

The nutrients of the three silages if calculated on an equivalent basis of 75·8 per cent. moisture are:

	Starch equivalent %	Digestible protein %
Tower grass silage	12·6	0·9
Heap grass silage	9·3	0·5
Pit grass silage	9·9	1·1

The grass silage made in the tower is definitely superior in starch equivalent value to both the other silages and better, as regards digestible true protein, than grass silage made in the heap and but little inferior to pit silage in this latter respect.

If the nutrients in the tower silage are compared with those in the hay, on a basis of equivalent dry matter, the following values are obtained:

	Starch equivalent % dry matter	Digestible true protein % dry matter
Tower grass silage	52·1	3·7
Average meadow hay	36·2	4·4

The dry matter of the grass silage is definitely superior to that of average meadow hay as far as starch equivalent is concerned and not far behind in digestible true protein content. This shows that it is possible to make a feeding stuff of high nutritive value in a tower silo.

<div align="center">Feeding trial.</div>

In order to test the silage under practical conditions, it was decided to feed the material made in the tower silo to a selected group of dairy cows. A group of six cows varying in yield from 1½ to 5 gallons per day was selected.

The ration fed to this group was as follows:

Maintenance ration. 35 lb. mangolds ⎱ per head daily.
15 lb. hay ⎰

Production ration. 3½ lb. of a balanced dairy cube per gallon of milk produced.

This ration was fed to all cows over the period February 10 to March 9 inclusive.

On March 10 the grass silage was introduced into the maintenance ration, the production ration being kept unchanged. The maintenance ration was altered to 35 lb. mangolds and 35 lb. of grass silage, the change being made over a period of three days. The production ration was still fed at the rate of $3\frac{1}{2}$ lb. per gallon of milk produced, the silage period lasting till April 6. The quantities of silage and hay fed would supply approximately equivalent amounts of starch equivalent.

The total yields of the cows and the total yield of the group during the two periods are given below.

Table VII. *Milk yield of cows (in lb.).*

	Root and hay period February 10–March 9	Root and silage period March 10–April 6
Daisy May	$362\frac{1}{4}$	$342\frac{1}{4}$
Mollie	$436\frac{1}{4}$	$420\frac{3}{4}$
Norah	$807\frac{1}{4}$	832
Lovely	$446\frac{3}{4}$	$484\frac{1}{2}$
Fanny	1179	$1074\frac{1}{4}$
Maggie	$907\frac{3}{4}$	$915\frac{1}{4}$
Total	$4139\frac{1}{4}$	$4069\frac{3}{4}$

The figures show that without exception the cows have milked well on the silage ration and that there has been a tendency for the milk yield to increase.

This is shown more clearly in Fig. 2, in which the total and individual daily yields are plotted, in terms of pounds of milk.

It is obvious from this that the grass silage has been as efficient as the hay and has definitely checked the rate of fall of the milk yield.

THE DIGESTIBILITY AND COMPARATIVE YIELD OF ARTIFICIALLY DRIED GRASS FROM THE SAME SOURCE.

At the same time as the grass was being filled into the silo, every third load was dried on a band drier with an inlet air temperature of about 200° C.

The analysis of the artificially dried grass is given in Table VIII together with the analysis of the original grass and the silage made from it. The average values for the contents of the two sacks embedded in the silo have been taken for the latter figures. The loads taken for drying were not sub-sampled so that this is the only measure of comparison, and this is made on a basis of the composition of the dry matter.

Table VIII. *Composition of original grass, grass silage and artificially dried grass (stated as percentage of dry matter).*

	Original grass	Grass silage	Dried grass
Ether extract	3·46	4·33	2·35
Fibre	23·99	28·28	25·38
Crude protein	13·93	14·78	14·55
Ash	7·86	9·90	8·29
Nitrogen-free extractives ...	50·76	42·71	49·43
	100·00	100·00	100·00
Organic matter	92·15	90·10	91·71
True protein	11·85	7·82	12·58
Lime (CaO)	0·61	0·77	0·70
Phosphoric acid (P_2O_5) ...	0·73	0·63	0·66
Ratio true : crude protein ...	0·85	0·53	0·86
Moisture in original material	74·9	74·7	15·0

With the exception of the ether extract there is good agreement between the dry-matter constituents of the original and the artificially dried grass. If due allowance be made for experimental error it may be said that the drying process has in no way affected the composition of the grass.
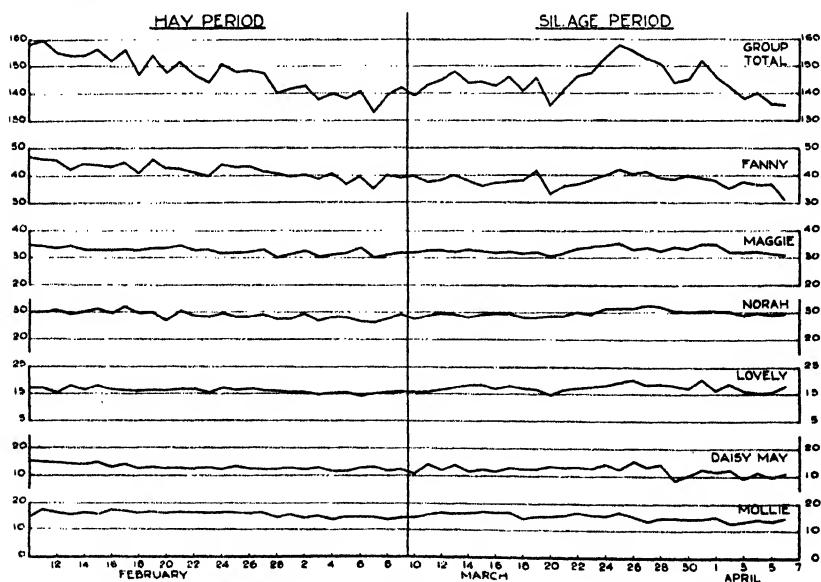
Fig. 2.

A digestibility trial was carried out on the dry material obtained, two Kerry cross wethers being used for this purpose. The technique of the trial was the same as that employed for the grass silage trial except that

all the feeds were weighed out on one day prior to the commencement of the trial. A pre-experimental period of four days and an experimental period of eight days were employed. 900 gm. of dried grass were fed daily and this amount was eaten readily, there being no residues.

The digestibility coefficients, calculated from the usual data, which are given in the appendix, are tabulated below.

Table IX. *Digestibility coefficients of artificially dried grass.*

	Sheep C	Sheep D	Average
Dry matter	69·51	73·74	71·63
Organic matter	71·66	75·61	73·64
Ether extract	47·92	54·17	51·05
Fibre	74·76	80·42	77·59
Nitrogen-free extractives	72·50	76·07	74·29
Crude protein	67·53	66·63	67·08
True protein	67·14	68·57	67·86

The agreement between the sheep is satisfactory though somewhat greater than is usual. If compared with the values for the grass silage it will be noticed that there is a marked similarity in the digestibility coefficients.

Table X. *Digestibility coefficients of artificially dried grass and grass silage made in a tower.*

	Grass silage %	Dried grass %
Dry matter	71·74	71·63
Organic matter	73·02	73·64
Ether extract	77·68	51·05
Fibre	77·26	77·59
Nitrogen-free extractives	71·73	74·29
Crude protein	67·70	67·08
True protein	49·55	67·86

The two points of difference are the figures for ether extract and the true protein. The higher digestibility of the former in the silage is readily understood when it is realised that this constituent includes the organic acids formed as a result of the fermentation processes, all of which are soluble and thus completely digestible. It has been noticed before in this laboratory that there is usually a depression of the digestibility of the true protein during the making of ensilage. This does not appear to arise in the process of drying grass artificially.

The digestible nutrients in the dried grass are given in Table XI, together with the values for grass silage made in a tower.

Table XI. *Digestible nutrients in artificially dried grass and grass ensilage made in a tower silo.*

	Artificially dried grass	Tower grass silage
Moisture in original material ...	15·0	75·8
Digestible crude protein	8·3	2·4
Digestible ether extract	1·0	0·8
Digestible nitrogen-free extractives	31·2	7·4
Digestible crude fibre	16·7	5·3
Digestible true protein	7·2	0·9
Starch equivalent per 100 lb. ...	44·1	12·6

If these be compared on a dry matter basis the nutrients are as follows:

	Starch equivalent %	Digestible protein %	
		Crude	True
Grass silage	52·1	9·9	3·7
Artificially dried grass	51·9	9·8	8·5

There is no difference in the starch equivalent and digestible crude protein contents of the two foodstuffs, but the silage shows a diminution in digestible true protein content.

The comparative yield of nutrients obtainable by these two processes can be calculated. The figures in Table XII give the result of three tests of the effect of artificial drying on the yield of dry matter.

Table XII. *Weights of dry matter in the original grass and the artificially dried material produced from it.*

	Fresh grass			Dried grass		
Test no.	Weight lb.	Moisture %	Dry matter lb.	Weight lb.	Moisture %	Dry matter lb.
1	819	81·7	150	237	36·4	151
2	397	76·2	94·5	123	22·4	95·5
3	720	75·3	178	190	9·6	172

It is evident that there is no loss of dry matter as a result of the drying process.

The loss of weight due to the process of ensilage (Table I) is 11·7 per cent.; the average of 14·4 and 8·9 per cent. The figures for dry matter in Table III give a value of 11·3 per cent., the average of 6·0 and 16·5 per cent., for the loss resultant on ensiling. 1000 lb. of fresh grass, with a moisture content of 75 per cent., would produce 883 lb. of silage, with a moisture content of 75 per cent., which would contain the nutrients quoted below in Table XIII.

Similarly 1000 lb. of fresh grass with a moisture content of 75 per cent.

would produce 294 lb. of artificially dried grass, moisture content 15 per cent., containing the following amounts of nutrients.

Table XIII. *The nutrients contained in the produce from 1000 lb. of fresh grass ensiled and artificially dried.*

	Produce from 1000 lb. fresh grass			
	Weight lb.	Starch equivalent lb.	Digestible crude protein lb.	Digestible true protein lb.
Tower grass silage ...	883	111·2	21·2	7·9
Artificially dried grass	294	129·6	24·4	21·2

The advantage is slightly in favour of the artificially dried grass as far as the starch equivalent and digestible crude protein are concerned, but markedly so in the case of the true protein. It should be realised, however, that the artificially dried grass is superior to good meadow hay which generally contains 31 per cent. of starch equivalent and 4 per cent. of digestible true protein as compared with 52 and 8·5 per cent. respectively in the artificially dried grass, and that grass silage would more usually replace ordinary hay in the ration and not artificially dried grass.

SUMMARY.

It is possible to make ensilage of high digestibility from grass in a tower silo. The loss by drainage is negligible and can in all probability be avoided entirely.

The losses due to fermentation are not high and the final product is a highly nutritious foodstuff.

The fact that all the silage with the exception of the uppermost layer, which was not expected to be used for feeding but merely as a protective layer, has been fed and was eaten readily is proof of the ease with which grass silage of high feeding value can be made in a tower silo if adequate precautions are taken.

Dairy cows will eat grass silage readily and the milk yield is not affected when the silage replaces the whole of the hay in the maintenance ration.

The artificial drying of grass results in the production of a material of high digestibility. The amounts of starch equivalent and digestible crude protein, obtainable in the silage and artificially dried grass produced from 1000 lb. of fresh grass, are somewhat higher in the case of the dried grass. The digestible true protein is considerably lower in the grass silage.

In general practice the silage would, however, be a substitute for ordinary hay and not an artificially dried grass. It should be noted that the silage was made from grass which had reached a stage when it might have been cut for early hay and when some of the earlier grasses were showing a "head."

ACKNOWLEDGMENTS.

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APPENDIX I.

Analysis of silage effluent.

Sample	1, 2, 3	4	5	6	7	8	9	10	11	12	13	14	15	Total weight in effluent 624 litres	
Dates	21/6–10/7 1929	22/7–6/8 1929	9/8–16/8 1929	19/8–27/8 1929	2/9–10/9 1929	16/9–30/9 1929	9/10–25/10 1929	1/11–19/11 1929	26/11–3/12 1929	6/12–13/12 1929	16/12–23/12 1929	27/12–31/12 1929	2/1–8/1 1930	gm.	lb.
Dry matter %	4·49	5·65	5·75	6·22	6·22	6·49	6·51	6·64	6·55	5·67	5·25	5·59	5·37	36,770	81
Ash %	1·57	1·78	1·90	1·81	1·91	1·79	1·80	1·77	1·71	1·56	1·41	1·44	1·45	10,512	23
Total N %	0·276	0·360	0·390	0·396	0·380	0·394	0·381	0·373	0·346	0·312	0·299	0·292	0·280	2,150	4·7
Ammoniacal N %	0·180	0·164	0·117	0·125	0·131	0·125	0·138	0·118	0·099	0·092	0·089	0·079	0·085	740	1·6
Amino N %	0·098	0·184	0·223	0·115	0·243	0·171	0·190	0·217	0·197	0·184	0·171	0·144	0·158	1,102	2·4
Volatile acids as acetic acid %	0·986	1·129	1·006	0·875	1·044	1·213	1·073	1·270	1·242	1·129	1·073	1·016	0·988	6,741	14·8
Acidity of alcohol extract. No. c.c.≡1 c.c. N/1 acid	4·18	3·14	2·99	4·10	3·05	2·84	3·05	2·67	2·40	2·74	2·84	2·84	2·97	—	—
Undetermined N %	—	0·012	0·050	0·156	0·006	0·098	0·053	0·038	0·050	0·036	0·039	0·069	0·037	308	0·7

APPENDIX II.

Digestibility coefficients of grass silage.

Sheep A	Dry matter gm.	Organic matter gm.	Ether extract gm.	Fibre gm.	Nitrogen-free extractives gm.	Crude protein gm.	True protein gm.
Silage eaten	6833	6157	296	1932	2918	1010	534
Faeces	1943	1670	69	459	821	324	263
Weight digested	4890	4487	227	1473	2097	686	271
% digested	71·57	72·88	76·69	76·24	71·86	27·92	50·75
Sheep B							
Silage eaten	6931	6245	300	1960	2960	1024	542
Faeces	1946	1676	64	426	841	333	280
Weight digested	4985	4569	236	1534	2119	691	262
% digested	71·91	73·16	78·67	78·27	71·59	67·48	48·34
Average	71·74	73·02	77·68	77·26	71·73	67·70	49·55

APPENDIX III.

Daily milk yield of cows.

Date	Root and hay period

Date	Daisy May	Mollie	Norah	Lovely	Fanny	Maggie	Total
February 10	15½	14¾	29¾	17	47	34½	158½
February 11	15	17½	30	17	46	34¼	159¾
February 12	14¼	16	30¾	15½	45¼	33½	155½
February 13	14¼	15½	29½	18	42½	34¼	154¼
February 14	14	16¼	30	16¾	44½	33	154¼
February 15	14¾	15¾	31¼	18	44	32¾	156½
February 16	13	17	29½	16½	43½	32¾	152¼
February 17	14	16¾	32	16	44¾	33	156¼
February 18	12¼	16	29½	15½	41	32¾	147
February 19	13	16¼	29¼	16	46	33½	154¾
February 20	12½	16	27	16	42¾	33½	148
February 21	12¾	16¼	30¼	16	42¾	34½	152½
February 22	12¼	16¼	38½	16½	41¼	32½	147¼
February 23	12¾	16	28	15¼	40	32¾	144¾
February 24	12¼	16¼	29¼	17	44½	31¼	151¼
February 25	13¼	16¼	28	16	43¼	31½	148¼
February 26	12½	16	28½	16½	43½	31½	149
February 27	12	16¼	29	16	41¾	33	148
February 28	12	14¾	27½	15½	41	30	140½
March 1	12½	15½	27½	15½	39¾	31¼	142
March 2	12	14¾	29¼	15¼	40¼	32	143½
March 3	12¾	15	27	14½	39	30¼	138½
March 4	11¾	13¾	28	15	41	31	140¼
March 5	11¾	14¾	28	15¼	37½	32	138½
March 6	12¼	14¾	26¼	14	40	33½	141¼
March 7	13	14¼	26	14¼	35¼*	29¾	133¼
March 8	11½	13¾	27½	15½	40¼	31	140
March 9	12½	14¼	29	15¾	39½	31½	142½
Total	362¼	436¼	807¼	446¾	1179	907¾	4139¼

* Served.

APPENDIX III (*continued*).

Root and silage period

Date	Daisy May	Mollie	Norah	Lovely	Fanny	Maggie	Total
March 10	10¼	14½	27½	15½	40	31¾	139¾
March 11	14	15½	28½	15½	37¾	32¼	143¼
March 12	12	16¼	29¼	16¼	38¾	32¼	145¼
March 13	14	16	29	17	40½	32	148¼
March 14	11¼	16	27¾	18	38½	33	144½
March 15	12¼	16¾	29	18	36½	32¼	144½
March 16	11½	16¼	29½	16¾	37¾	31½	143¼
March 17	12¾	16½	29¼	18	38	32	146¾
March 18	12½	14¼	28	17	38½	31½	141¼
March 19	12½	15	28	16¾	42	32	146½
March 20	13¼	15	28½	14¾	33¾*	30¼	135¾
March 21	13	15½	28¼	16¼	36¼	31¾	141¼
March 22	13	16¼	30	17	37	33¼	146½
March 23	12¾	15½	29¾	17¾	38½	34	148¼
March 24	14¼	15	31¼	18	40¼	34¼	153¼
March 25	12¾	16¼	31¼	19½	42¼	35¼	158
March 26	15¼	15	31½	20	41	33	156
March 27	13½	13½	32¾	18¼	41¾	33¾	153¼
March 28	14¼	14¼	32	18¾	39¼	32¼	151
March 29	8¼	14¾	30¼	18	38¾	34	144¼
March 30	10½	14½	30	17	40	33¼	145¼
March 31	12¼	14½	30¾	20½	39¼	35	152½
April 1	11½	15	30½	16	38¼	35	146¼
April 2	12¼	13	30½	18¾	35¾	32¼	142¾
April 3	9½	13	29¼	16¼	38	32	138
April 4	11¼	14¼	30	15½	37	32½	140½
April 5	9½	13½	29	15¼	37	31½	136¼
April 6	11	14½	29¾	18	31¼	31	135¾
Total	342¼	420¾	832	484½	1074¾	915½	4069¾

* Served.

APPENDIX IV.

Digestibility coefficients of artificially dried grass.

	Dry matter gm.	Organic matter gm.	Ether extract gm.	Fibre gm.	Nitrogen-free extractives gm.	Crude protein gm.	True protein gm.
Sheep C							
Grass eaten	6120	5613	144	1553	3025	890	770
Faeces	1866	1591	75	392	832	289	253
Weight digested	4254	4022	69	1161	2193	601	517
% digested	69·51	71·66	47·92	74·76	72·50	67·53	67·14
Sheep D							
Grass eaten	6120	5613	144	1553	3025	890	770
Faeces	1607	1369	66	304	724	297	242
Weight digested	4513	4244	78	1249	2301	593	528
% digested	73·74	75·61	54·17	80·42	76·07	66·63	68·57
Mean	71·63	73·64	51·05	77·59	74·29	67·08	67·86

THE ANALYSIS OF TOMATO PLANTS. PART II.

THE EFFECT OF MANURIAL TREATMENT ON THE COMPOSITION OF TOMATO FOLIAGE.

By O. OWEN.

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(With Four Text-figures.)

THE typical effects of nitrogen and potash deficiencies respectively on tomato foliage and the apparent absence of any effect due to the omission of phosphates from the manures suggested that the analysis of foliage from plants grown under these conditions would prove of interest. The present paper deals with such analyses and the data obtained confirm two suggestions made in an earlier paper (8). These were, firstly, that where the potash concentration in the soil is low, the percentage phosphoric acid in the plants is high, and secondly, that where the phosphoric acid concentration in the soil is low, the percentage potash in the plants is low.

The samples of foliage analysed were taken from plants growing in plots which had been treated continuously in the same way for some years. Since 1916 the manurial treatment had consisted of complete artificials (plot 5), and complete artificials without phosphates (plot 6), without nitrogen (plot 7) and without potash (plot 8) respectively. The leaves analysed were taken from a number of plants (variety Manx Marvel) during June, except in 1927 when the samples were taken during August. To make the samples comparable the leaves were taken from near the fifth fruit trusses except in plot 7 where, owing to the limited growth of the plants, they were taken from all parts of the plants.

The samples were prepared and analysed as already described.

The results are shown in Table I.

The omission of phosphates or nitrogen appears to have no consistent effect on the ash, but in each of the four years the omission of potash depresses the ash content.

THE EFFECTS ON THE NITROGEN CONTENTS.

It will be noticed that the values for nitrogen in the various years rise and fall together, that is to say, the nitrogen content of the foliage is influenced by seasonal variation irrespective of manurial treatment. This

is of interest in view of the results published by Bewley[1] to show that crop weight in tomatoes follows seasonal changes irrespective of manurial treatment.

Table I. *The percentage composition of dried tomato foliage.*

Plot and treatment	1924	1925	1926	1927
	Ash.			
5. Complete artificials	32·53	26·97	34·47	30·40
6. Complete artificials without phosphates	36·90	25·32	32·59	34·45
7. Complete artificials without nitrogen ...	30·55	27·69	35·43	33·28
8. Complete artificials without potash ...	31·32	25·36	27·57	25·01
	Nitrogen.			
5. Complete artificials	3·914	3·908	3·077	5·141
6. Complete artificials without phosphates	3·966	3·700	2·465	4·331
7. Complete artificials without nitrogen ...	2·393	2·210	1·737	2·905
8. Complete artificials without potash ...	3·594	3·668	2·639	5·028
	Potash (K_2O).			
5. Complete artificials	6·405	5·710	8·734	7·273
6. Complete artificials without phosphates	3·753	4·661	6·990	5·592
7. Complete artificials without nitrogen ...	6·676	6·670	7·214	3·580
8. Complete artificials without potash ...	2·454	1·192	1·180	1·891
	Phosphoric acid (P_2O_5).			
5. Complete artificials	0·7307	0·6817	0·6992	0·5577
6. Complete artificials without phosphates	0·6683	0·5589	0·6103	0·5358
7. Complete artificials without nitrogen ...	0·4645	0·6683	0·6592	0·5538
8. Complete artificials without potash ...	0·9959	0·6717	0·9506	0·9436

The omission of phosphates had little effect on the nitrogen content in 1924 but depressed it in 1925, 1926 and 1927. The fall in the values in these years is noteworthy in that there was no apparent evidence of nitrogen starvation in the plants.

The omission of nitrogen caused a considerable depression in the nitrogen values and the low values can be associated with the marked effect on the appearance of the plants grown under these conditions.

The omission of potash does not appear to cause much difference in the nitrogen contents and this is in accord with the appearance of the foliage of potash-starved plants, which, in those parts not showing typical symptoms of potash starvation, exhibit a deep green colour in decided contrast to the pale appearance of the foliage of nitrogen-starved plants.

THE EFFECTS ON THE POTASH CONTENTS.

The annual fluctuations in nitrogen values are not accompanied by corresponding fluctuations in the potash values. The appearance of the plants would suggest that the omission of nitrogen has such a pronounced effect on the functions of the plants generally that they would show abnormal potash values. It will be seen from the table, however, that only

in 1927 did the omission of nitrogen cause a decided depression in the potash content. There is an appreciable depression in 1926, but in 1925 the value shows a definite increase and that for 1924 shows a smaller increase. Thus, it would appear that nitrogen manuring is variable in this respect or, more probably, that its effect is such that it is considerably modified by other factors.

The effect of the omission of potash is clearly shown in the figures for the foliage from this plot. In the four years the potash contents are reduced to approximately less than half, a fifth, a seventh and a quarter respectively, of those for the foliage of plants receiving complete artificials.
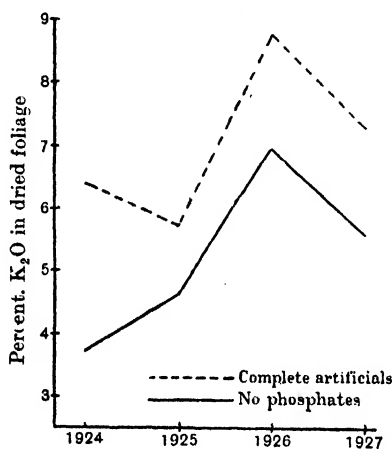
Fig. 1.

The figures of greatest interest in this section are those for the plot from which phosphates have been withheld. These are depicted in Fig. 1. It will be seen that in each of the years the potash contents of the foliage are lower where phosphates were omitted than where complete artificials were applied, despite the fact that the same amount of potash had been applied to each of these plots each year. In other words tomato plants do not take up so much potash where phosphates are withheld continuously from the soil.

A point which may be raised here is that these plants fail to show those symptoms which usually accompany potash deficiency. These symptoms include a high susceptibility to disease, particularly "stripe." Further, an unprofitably high proportion of fruit shows blotchy ripening and thirdly, the foliage shows leaf scorch. In the no-phosphate plots here none of these effects appears, and it seems probable that they do not

occur unless the potash level in the plant is below a certain level. Under the conditions prevailing this minimum has never been reached.

Since the omission of phosphates from manures depressed the potash content of the foliage, it appeared reasonable to suppose that addition of phosphates to a phosphate-starved soil would increase the potash content of the foliage. This view was tested in 1927 for plots receiving graded amounts of phosphates. In 1923 plot 6 was divided into four sub-plots. All four received the same amounts of potassic and nitrogenous fertilisers, but the amounts of phosphates added were varied, phosphates always being withheld from sub-plot 6 a. For the 1926 and 1927 seasons the percentages of phosphates added in the whole dressings (base and top) amounted to approximately 0 (6 a), 6·3 (6 b), 10·4 (6 c) and 13·35 (6 d) calculated as $Ca_3(PO_4)_2$. The potash contents of the foliage in 1927 under these conditions are shown in Fig. 2.
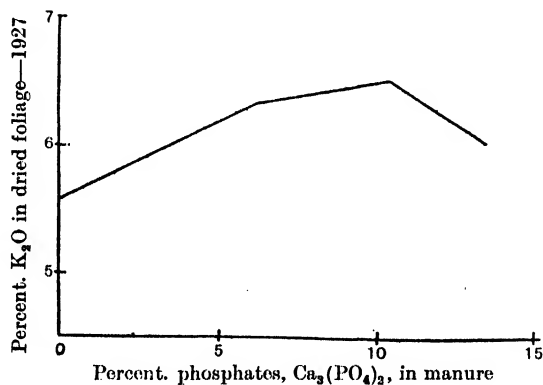
Percent. phosphates, $Ca_3(PO_4)_2$, in manure
Fig. 2.

It will be seen that the addition of the phosphates is accompanied by a decided increase in the percentage potash in the foliage. The increase is progressive up to 10·4 per cent. phosphates in the fertiliser, but at 13·35 per cent. phosphates the potash value is less than at the other two concentrations of phosphates. This may be due to the operation of some process akin to the Law of Diminishing Returns. On the other hand seasonal factors may be responsible. Unfortunately it was not possible to re-examine the question during 1928, as it was necessary to steam the soils in the 1927–8 winter. The data for the following season, while of interest, do not concern the present work. In any case it seems to be definite that where the phosphoric acid concentration in the soil falls below a certain value the potash absorbed by the tomato plant is reduced irrespective of an adequate potash supply.

While this paper was in preparation it was suggested to the author that although the results were valid for percentage composition on a dry matter basis, the total amount of potash removed by the crop might not be influenced by the amount of phosphates applied to the soil. As the analyses of the total crops have been impossible this question cannot be answered. The criticism can be partly met, however, by the following figures. They are for samples of mature leaves taken in September, 1930, and the potash is referred to a *green* weight basis in each case.

Complete artificials without phosphates   0·5419 % K_2O
Complete artificials   ...   .   0·6259 % K_2O

The difference on this basis is of the same order as that recorded on the dry matter basis.

THE EFFECTS ON THE PHOSPHORIC ACID CONTENTS.

As was to be expected the omission of phosphoric acid from the manures is accompanied by a reduction in the phosphoric acid content of the tissue in each of the four years, the reduction varying from about 3·6 to 18 per cent. on the values of the foliage of plants grown with complete artificials.

Only in one year (1924) was the omission of nitrogen accompanied by a decided reduction in the phosphoric acid content. This may mean that the omission of nitrogen is without appreciable effect on the absorption of phosphate per unit tissue, although, of course, the total phosphate absorbed by the plants is considerably reduced since the total weight of the plants under these conditions is much lower than normal.

The figures of greatest interest in this section are those for the plot receiving no potassic fertilisers. These are shown in Fig. 3.

Except in 1925 when values for "no-potash" and "complete artificial" foliage were almost identical the omission of potash is followed by a definite increase in the phosphoric acid content of the foliage. It may be remarked that the value of 0·9959 per cent. P_2O_5 recorded for foliage from the no-potash plot in 1924 is the highest obtained during the analysis of well over a hundred samples of mature leaves during the course of this work. The point which next arose, namely, whether addition of potash would depress the phosphoric acid content of the foliage, was examined in 1927 when samples of foliage from four sub-plots receiving different amounts of potash were analysed. Briefly the history of the sub-plots is as follows. The original no-potash plot (8) was divided into four sub-plots for the 1923 season. Since then they have received equal amounts of

nitrogen and phosphate each year, but the potash applied was varied, potash being withheld from one sub-plot (8 *a*) each year. For 1927 the whole dressings contained sulphate of potash corresponding to 2·72 per cent. (8 *b*), 3·56 per cent. (8 *c*) and 5·33 per cent. (8 *d*) K_2O respectively.
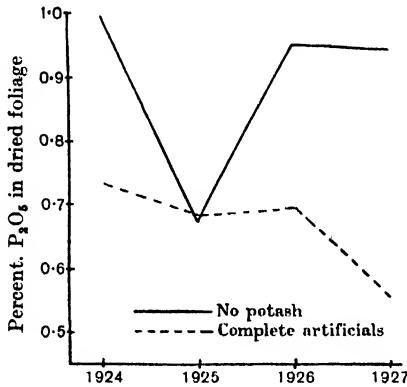
Fig. 3.
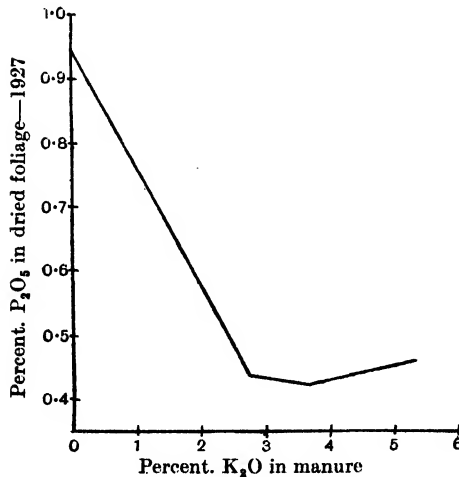
Fig. 4.

The phosphoric acid contents of the foliage from these plots are shown in Fig. 4.

From the figure it will be seen that the lowest amount of potash applied in the dressing reduces the percentage phosphoric acid in the foliage to less than one-half of that of the foliage of plants in the plot receiving no potash. It appears to be established, therefore, that in the

absence of an adequate supply of potash in the soil the tomato plant will take up more phosphate than it does where the potash supply is adequate. It is not suggested that phosphoric acid will replace potash. On the contrary, it is obvious that other bases will be absorbed in the absence of sufficient potash, and, while we have no data on this point for the tomato, results of other workers indicate that for some plants at all events increase in the phosphoric acid content when the potash content is low is accompanied by an increase in magnesia and lime.

Table II. *The percentage composition of dried tomato foliage*, 1927.

Plot and treatment	Nitrogen	Phosphoric acid (P_2O_5)	Potash (K_2O)
5. Complete artificials	5·141	0·5577	7·273
6 a. Potash and nitrogen; no phosphates ...	4·331	0·5358	5·592
6 b. Potash and nitrogen; 6·3 % phosphates	4·193	0·6604	6·351
6 c. Potash and nitrogen; 10·4 % phosphates	4·567	0·6443	6·520
6 d. Potash and nitrogen; 13·35 % phosphates	4·743	0·9399	6·040
7 a. Potash and phosphates; no nitrogen ...	2·905	0·5538	3·580
7 b. Potash and phosphates; 1·09 % nitrogen	4·629	0·5697	5·416
7 c. Potash and phosphates; 1·55 % nitrogen	4·871	0·5291	5·751
7 d. Potash and phosphates; 2·18 % nitrogen	5·660	0·6685	6·061
8 a. Phosphates and nitrogen; no potash ...	5·028	0·9436	1·891
8 b. Phosphates and nitrogen; 2·72 % potash	3·841	0·4414	4·163
8 c. Phosphates and nitrogen; 3·56 % potash	4·049	0·4254	4·658
8 d. Phosphates and nitrogen; 5·33 % potash	4·334	0·4648	5·069

DISCUSSION.

From an examination of the literature it appears that this interdependence between soil potash and phosphates on the one hand and the percentages of phosphate and potash in the foliage of plants on the other is not confined to the tomato plant alone, although much of the evidence is of an indirect nature.

Wallace in a series of papers on fruit trees and bushes supplies a considerable amount of data in support of the views now presented. Calculated to a fresh-weight basis he finds that when the manurial treatment does not include phosphorus the percentage of potash in gooseberry leaves [9] is lower than where organic phosphorus is applied, but somewhat higher than where inorganic phosphorus is included. In the stems the potash content is lower in the absence of phosphorus than it is where either organic or inorganic phosphorus is applied. Where potash is omitted the phosphoric acid contents of both stems and leaves are definitely greater than where potash is applied. In the case of plums [10] low values for the potash in the ash of chlorotic foliage are accompanied by higher

phosphoric acid values than in green leaves where the potash is high. In a more recent paper on apples(11) the same author shows that where potash was omitted the percentage phosphoric acid in the dry matter of leaves, stems and petioles, and wood and bark is lower than that in the corresponding parts of trees receiving complete nutrient. In an account of experiments on the control of leaf scorch of red currants and gooseberries(12), Wallace gives some interesting figures for these plants after treatment with potash. Leaves and stems of gooseberries which had reacted satisfactorily to the treatment showed a decided increase in potash content compared with leaves and stems of control bushes, while there was a decided fall in the phosphoric acid content under the same conditions. Red currants did not respond in the same way and Wallace suggests that the plants had not been able to utilise the potash supplied. It may be noted that in the case of the red currants there was very little fall in the percentage of phosphoric acid in the dry matter of the leaves and stems where potash had been applied.

Similar results were obtained for the vine by Lagatu and Maume(4). Vines were grown under different manurial conditions and two leaves from the base of fruiting shoots were analysed monthly from May 16 to October 16, 1925. For the leaves examined in May and June the omission of phosphates is accompanied by an increase in the potash content, but the remainder of the samples show a decreased percentage of potash when phosphates are omitted. With one exception all the samples show an increased phosphoric acid content when potash is omitted from the manure.

Among the older papers several give support to the present conclusions. Lawes and Gilbert(5) show that for gramineous, leguminous and miscellaneous herbage during four years in the seventies the omission of potash is followed by an increase in the phosphoric acid content of the pure ash. The mean values are 7·32 per cent. P_2O_5 with potash and 8·76 per cent. in its absence. Similar results are given for various years between 1856 and 1873, the greatest difference occurring for the three years 1866–8 when the pure ash contained 9·98 per cent. and 6·23 per cent. P_2O_5 without and with potash respectively. The same authors show that the same applies to wheat straw(6) for the years 1852–71. In every case both for the ash and the dry matter the omission of potash is accompanied by an increase in the phosphoric acid content. Data given by Hall(3) indicate that this holds also for Barnfield mangels but not for Hoosfield barley.

Apart from any light these conclusions may throw on the rôle of mineral elements in plant nutrition, they have an important bearing on

the use of plants and foliage as a means of determining manurial requirements. It is clear that where foliage shows symptoms ordinarily associated with, say, potash deficiency the addition of phosphates may be necessary to enable the plant to make full use of any potash applied. Again, where the available potash or phosphate is determined by analysis of seedlings grown in the soil as in the Neubauer method, care must be exercised in the interpretation of the results. If the conclusions reached in this paper are of general application the availability of potash in soil is considerably influenced by the phosphorus status of the soil, and the amount of phosphorus taken up is conditioned by the potash status of the soil. In this connection a paper by Neubauer, Bonewitz and Schott-Mueller is of interest[7]. These authors show that fertilisation with superphosphate increases the utilisation of added potash, while basic slag depresses it. Addition of potassium chloride with superphosphate or basic slag depresses the root-soluble phosphoric acid as compared with its solubility with either source of phosphorus but without the potassium chloride.

One other point remains to be dealt with. Table II gives the full analytical data for the 1927 samples. It will be seen from the figures for the sub-plots 8 *a*, 8 *b*, 8 *c* and 8 *d* that, roughly speaking, phosphoric acid in the foliage falls as the potash rises. Data for tomato fruit given in a previous paper[8] suggest that in the fruit there is a direct correlation between potash and phosphoric acid. This is interesting in that Miss Brown[2] has shown that there is a direct correlation between potash and phosphate in apples, while Wallace[11] has shown that where the potash content of apple leaves falls the phosphoric acid rises. Similarly the data of Lawes and Gilbert[6] show that in wheat grain potash and phosphoric acid rise and fall together, while in the straw increase in potash content is accompanied by a fall in phosphoric acid content.

Summary and Conclusions.

Analyses of foliage of tomato plants grown under different manurial conditions are recorded for the four seasons 1924–7.

The nitrogen content of the dried foliage shows a seasonal variation irrespective of manurial treatment.

Where phosphates are omitted from the manure the potash content of the foliage is depressed. When phosphates are added to a soil from which they had previously been withheld the potash content of the foliage is increased.

Where potash is omitted from the manure the phosphoric acid of the

foliage is considerably increased. When the deficiency of soil potash is rectified the phosphoric acid content of the foliage is reduced.

Whereas there is an indirect relationship between potash and phosphoric acid in tomato foliage it is probable that in the fruit the relationship is direct.

It is suggested that these conclusions apply to some other plants, and their bearing on the use of foliage and plants as indicators of manurial requirements is briefly discussed.

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INVESTIGATIONS INTO THE INTENSIVE SYSTEM OF GRASSLAND MANAGEMENT.

By the Agricultural Research Staff of Imperial Chemical Industries, Limited.

VI. THE DIGESTIBILITY AND FEEDING VALUE OF GRASS SILAGE MADE IN A STACK.

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INTRODUCTION.

THE preservation of grass in the form of silage is of particular interest since every grass farmer is faced with the problem of conserving his surplus grass at certain times of the year.

During the months of August and September such a surplus often exists and it is often impossible to make it into hay owing to unsuitable climatic conditions.

Researches have been carried out at Jealott's Hill on the making of grass silage and conservation of grass and this communication is one of the series of papers on this subject.

THE TREATMENT OF THE GRASS AND THE MAKING OF THE SILAGE.

Two samples of grass silage were obtained from Clitheroe in Lancashire during January, 1930. The silage was made in stacks from aftermath grass from old grassland which had received basic slag and potash for the hay, and nitrogen for both hay and aftermath.

The cutting of the aftermath was commenced on August 21, 1929 at which time it had had about 8–9 weeks' growth. The silage was made in round stacks, 15 ft. in diameter. The grass was built up to a height of 5 ft. on the first day when it was well trampled down and some heavy beams were placed on top. It was left for 48 hours, the beams were taken off and a layer of about 3 ft. of grass was put on daily, each addition being well trampled.

When all the grass was stacked, a layer of about 4 ft. of rushes and rough grass was placed on top. This was not done until after the stack

had settled, with the result that rain spoilt a layer of 6 in. on top of the stack.

As the prevalent wind was affecting the settling of the stack, a stack-sheet was placed round the windward side and prevented the wind blowing directly on to it. The result was that the degree of fermentation was equal throughout the stack and it settled evenly.

There was a certain amount of waste round the sides and ends of the stack which could have been lessened had the stack been "pulled" and this material thrown on to the top. Silage No. 1 seemed to be more relished by stock, though both were eaten readily.

Digestibility trials with sheep were carried out at Jealott's Hill, on two samples of silage.

The sheep ate their rations willingly, sample 2 being apparently relished as much as sample 1.

Composition of the silage.

Table I gives the analyses of the two samples. From this table it will be seen that little difference is apparent in the chemical composition of the dry matter.

Table I. *Composition of grass silage samples.*

	Silage 1		Silage 2	
	% Silage	% Dry matter	% Silage	% Dry matter
Moisture	78·05	—	74·15	—
Ether extract	0·98	4·46	1·13	4·36
Fibre	6·09	27·76	7·07	27·36
Crude protein	3·69	16·82	4·21	16·29
Ash	2·50	11·37	2·17	8·41
Nitrogen-free extractives	8·69	39·59	11·27	43·58
	100·00	100·00	100·00	100·00
Organic matter	19·45	88·63	23·68	91·59
True protein	2·97	13·54	3·33	12·89
Calcium oxide (CaO)	0·32	1·44	0·24	0·93
Phosphoric acid (P_2O_5)	0·18	0·83	0·15	0·58
Ratio $\dfrac{\text{True protein}}{\text{Crude protein}}$	0·80	0·80	0·79	0·79

The greatest difference is to be found in the mineral content, sample 1 being decidedly richer in ash, lime (CaO) and phosphoric acid (P_2O_5) content.

Digestibility of the silage.

The digestibility coefficients have been calculated in the usual way from the analytical data of the silage and faeces and are given in Table II.

Table II. *The digestibility coefficients of grass silage made in the stack (per cent.).*

Silage 1	Dry matter	Organic matter	Ether extract	Fibre	Nitrogen-free extractives	Crude protein	True protein
Sheep A	60·54	62·99	63·28	79·67	62·37	33·03	23·21
Sheep B	61·19	63·74	64·97	80·40	62·39	37·87	26·58
Average: A and B	60·87	63·37	64·13	80·04	62·38	35·45	24·90
Silage 2							
Sheep C	62·48	64·71	69·71	77·16	63·57	44·53	33·65
Sheep D	59·81	62·13	68·39	78·23	60·43	35·87	23·81
Average: C and D	61·15	63·42	69·55	77·70	62·00	40·20	28·73

The digestibility coefficients of the two samples are very similar, and it may be assumed that the differences lie within the limits of experimental error. The digestibility of the crude and true protein is interesting, the value being very low compared with the values for the other constituents. Woodman (1), working with rye-grass and clover silage, made in the stack, also obtained abnormal results for the digestibility of the crude protein, his results being of a still lower order, namely 12·2 per cent.

The digestibility of the crude fibre is particularly high, a fact frequently noticed when examining silage. In addition, the fibre content of the silage was low, which is an added advantage.

Feeding value of the silage.

The digestibility coefficients of the two samples of silage are given in Table III together with the values for other types of silage quoted by Woodman (1) and Kellner (2).

Table III. *Comparison of digestibility coefficients of various samples of silage.*

	Silage No. 1 from Clitheroe	Silage No. 2 from Clitheroe	Grass silage from heap* Kellner (2)	Grass silage from pit* Kellner (2)	Rye-grass and Clover stack silage Woodman (1)	Maize silage Kellner (2)
Organic matter	63·4	63·4	57	59	49·3	67
Crude protein	35·4	40·2	50	70	12·2	51
Ether extract	64·1	69·5	48	50	70·0	80
Nitrogen-free extractives	62·4	62·0	58	58	55·9	67
Crude fibre	80·0	77·7	60	58	53·8	71

* Digestibility coefficients calculated from the values for crude and digestible constituents.

The digestibility coefficients of the two samples of silage compare very favourably with other silages. There is, however, evidence of a

depression of the digestibility of the crude protein but it is interesting to note in this connection that Kellner gives a range of 22 to 67 with an average value of 51 for the digestibility of the crude protein in maize silage.

The crude fibre in the two samples dealt with is shown to be of a high order of digestibility. It is well known that the process of ensilage increases the digestibility of the crude fibre and in addition it is very likely that the effect of the nitrogenous dressing would be to retard maturity in the aftermath with a consequent reduction of the amount of lignification of the cellulose in the original grass.

In Table IV are given the digestible nutrients contained in the two samples of silage together with the values quoted by Kellner(2) for heap and pit silage. The figures for good and poor meadow hay quoted by the same author are included for comparative purposes.

Table IV. *The digestible nutrients in grass silage; poor and good meadow hay. (Calculated on a dry matter basis.)*

	Silage No. 1 %	Silage No. 2 %	(2) Grass silage from pits %	(2) Grass silage from heaps %	(2) Poor meadow hay %	(2) Good meadow hay %
Digestible crude protein	5·9	6·5	7·2	5·9	4·0	6·3
Digestible ether extract	2·9	3·0	2·1	4·1	0·6	1·2
Digestible nitrogen-free extractives	24·7	27·0	24·2	23·4	22·5	30·0
Digestible crude fibre	22·2	21·2	19·6	18·4	19·2	17·6
Digestible protein	3·4	3·7	4·6	2·2	2·9	4·4
Starch equivalent per 100 lb.	43·4*	44·8*	40·7	38·4	22·2	36·2

* The utilisation value, V, used in the calculation of the starch equivalent was taken as being 78.

It is evident from these figures that grass silage made in a stack can have a greater value, based on dry matter content, than poor meadow hay and so far as the starch equivalent is concerned will be of higher value than good meadow hay. The lower content of digestible protein is of little import under ordinary practical feeding conditions.

In the case of the dairy cow the maintenance ration is often supplied by 20 lb. of good meadow hay. Assuming a moisture content of 15 per cent., this would supply the following quantities of nutrients:

Dry matter	...	...	...	17·0 lb.
Starch equivalent...	...	...	6·15 ,,
Digestible protein...	...	...	0·75 ,,

A ration could be made up consisting of 35 lb. of grass silage (25 per cent. dry matter) and 8 lb. of good meadow hay which would contain:

	From silage lb.	From hay lb.	Total lb.
Dry matter	8·75	6·8	15·55
Starch equivalent	3·85	2·45	6·30
Digestible protein	0·31	0·30	0·61

The standard adopted for the maintenance requirement of an animal of 1000 lb. live weight is 6·0 lb. of starch equivalent containing 0·6 lb. of digestible protein[3]. Both rations satisfy this requirement, and the silage ration is satisfactory as regards the amount of dry matter, of which it contains less than the ration of hay alone.

Though no actual feeding tests were carried out with the silage, it was fed with satisfactory results to stock and formed a large proportion of their winter ration.

SUMMARY.

It may be concluded from this trial that the making of stack silage is a useful method of conserving surplus grass, particularly of the aftermath type, when good haymaking weather may not obtain. Though no actual feeding trials were carried out with the silage, it was fed to stock with satisfactory results and formed a large proportion of their winter ration.

ACKNOWLEDGMENTS.

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APPENDIX.

The digestibility coefficients of grass silage made in the stack.

	Dry matter	Organic matter	Ether extract	Fibre	Nitrogen-free extractives	Crude protein	True protein
Silage 1.							
Sheep A.							
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Silage eaten	4644	4116	207	1289	1839	781	629
Solid excreta	1832	1523	76	262	692	523	483
Weight digested	2812	2593	131	1027	1147	258	146
% digested	60·54	62·99	63·28	79·67	62·37	33·03	23·21
Sheep B.							
Silage eaten	4413	3911	197	1225	1747	742	598
Solid excreta	1713	1418	69	240	657	461	439
Weight digested	2700	2493	128	985	1090	281	159
% digested	61·19	63·74	64·97	80·40	62·39	37·87	26·58
Mean	60·87	63·37	64·13	80·04	62·38	35·45	24·90
Silage 2.							
Sheep C.							
Silage eaten	4768	4367	208	1305	2078	777	615
Solid excreta	1789	1541	63	298	757	431	408
Weight digested	2979	2826	145	1007	1321	346	207
% digested	62·48	64·71	69·71	77·16	63·57	44·53	33·65
Sheep D.							
Silage eaten	4432	4059	193	1213	1931	722	571
Solid excreta	1781	1537	61	264	764	463	435
Weight digested	2651	2522	132	949	1167	259	136
% digested	59·81	62·13	68·39	78·23	60·43	35·87	23·81
Mean	61·15	63·42	69·55	77·70	62·00	40·20	28·73

THE EFFECTS OF SUMMER GREEN MANURES ON THE AMMONIA AND NITRATE CONTENTS OF SOILS CROPPED FOR WINTER WHEAT.

AN EXAMINATION OF THE WOBURN GREEN MANURE PLOTS[1].

By T. J. MIRCHANDANI.

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(With Three Text-figures.)

THE purpose of the Woburn experiments on green manuring was defined by Dr J. A. Voelcker in 1892 in the following words [1]: "Following up the recently ascertained truths respecting the assimilation of the free nitrogen of the air by the leguminous plants, it was sought to ascertain whether by growing green crops of a leguminous nature and ploughing them in as a preparation for a corn crop, a better corn crop will result through the enrichment of soil by accumulated nitrogen than where a non-leguminous crop was grown." The results were surprising. After tares (vetches-*Vicia sativa*) the wheat crop was almost invariably poorer than after mustard (*Sinapis alba*) and, further, the yields on both sets of plots steadily declined. Summarising the results 35 years later, Dr Voelcker said in a contribution to a Rothamsted Conference on Green Manures(2): "Altogether it seems quite unaccountable that such miserable crops of wheat should follow the pursuit of what may ordinarily be good farming practice. It is quite evident that there must be some factor as yet unknown to us, which produces a result not only at variance with scientific deductions, but with practical experience generally, for without assuming some disturbing element of this nature, it is incomprehensible that liberal treatment such as these plots have received could result in the production of crops so meagre. Many have been the attempts I have made to find a possible explanation and many the suggestions put forward but none has so far been found to be tenable."

The Rothamsted Conference in 1927 revealed a number of other cases in which green manures, especially tares, had given disappointing results

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[1] This paper is abridged from a portion of a "Thesis approved for the Degree of Doctor of Philosophy in the University of London."

in the following wheat crop though in no other case have observations been continued long enough to give such complete crop failures as at Woburn.

The work to be described in this and the following papers was undertaken to test the hypothesis that the production of relatively large amounts of nitrate during autumn and winter and their loss by drainage lead to a deficiency of available nitrogen at a critical period for wheat in the following summer. Systematic analyses of the soils of the Woburn green manure plots and small scale experiments with top dressings of sodium nitrate were carried out in the seasons 1928–9 and 1929–30, and are described here. The results of laboratory and pot-culture experiments with a fuller discussion of the hypothesis are given in a later paper (3).

<div align="center">LANSOME FIELD GREEN MANURE PLOTS.</div>

In the original experiment in Lansome field a wheat and a green manure crop have been taken in alternate years with a few exceptions since 1892. Generally there were two green crops during the summer and both were ploughed in. The mean yields of wheat following green manures were 12·4 cwt. per acre after mustard and 8·6 cwt. per acre after tares. On two occasions when a second cereal crop was taken after the wheat there was no difference between the tares and mustard. Until 1917 there was a third plot with rape which gave similar results to the mustard plot. In 1917 the experiment was rearranged; the rape plot was abandoned and a new pair of mustard and tares plots and one with a summer fallow started. Mineral manures and lime applied to half of the plots gave slight increases in yield but did not alter the relative effects of the green manures. From 1917 to 1927 the wheat crops on each of these plots were miserably small; only two (1923 and 1925) were considered worth harvesting and these gave below 4 cwt. of grain per acre. The crop failure appeared complete until 1928–9, when the yield returned to a reasonable size (20·6 and 15·3 cwt. of grain per acre on the old tares and mustard plots respectively) and for the first time the tares gave a better crop than mustard.

<div align="center">STACKYARD FIELD GREEN MANURE PLOTS.</div>

In 1911 a second series of plots was started in Stackyard field. The two summer crops of green manures were consumed on the plots by sheep which received cotton cake at the rate of 3 cwt. per acre. In 1921 the rotation was interrupted by taking a second wheat crop on half of the plots for one year so as to provide both wheat and green manure crops

each year. In 1923 one-half of each plot was limed. There are thus 8 plots in the experiment in sets of 4 cropped alternately with wheat and green manures.

At the commencement the land was known to be in poor condition, but the first wheat crop (1912, 10·1 and 9·8 cwt. grain per acre after tares and mustard) though poor has never been equalled. The falling off in yield proceeded more rapidly on the tares plots than on the mustard plots and was still further accelerated by liming (average yield for 12 crops 4·8 and 5·4 cwt. grain per acre after tares and mustard respectively on the unlimed portions).

It happens almost invariably that during the winter months the wheat after tares appears more advanced and healthier than that after mustard, and that both of them compare very favourably with other wheat in more normal rotations. About May the wheat after green manures falls behind other wheat and in the first few rotations the failure occurred earlier on the wheat after tares. On several occasions during the thirty years the dry weights and nitrogen contents of the green crops were estimated from samples in both fields and showed that the tares contained more nitrogen. Soil analyses showed that the tares plots contained more nitrogen than the mustard plots in both fields.

WHEAT AFTER GREEN MANURES 1928–9.

The wheat crops of 1928–9 in the two fields differed in one important particular. On Lansome field the customary two successive green manure crops were grown during the summer (tares from May 14 to July 13, and from July 14 to October 28, 1928; mustard from May 30 to July 13, and from July 27 to October 27, 1928) and wheat was sown almost immediately (November 1). On Stackyard field, however, there was only one crop of each green manure (tares, sown May 16, folding July 31 to August 8, 1928 and mustard sown May 31, folding July 23 to July 30, 1928). From the beginning of August until November 6 the plots were bare fallowed. During these fallow periods there were 51 days with rain and a total rainfall of 6·75 in., with 6 extra rainy days and 1·06 in. extra rainfall after the folding of the mustard and during that of the tares. This high and well-distributed rainfall provided opportunity for nitrification and leaching out of some of the nitrate formed in Stackyard field, whereas on Lansome field much of the nitrate formed during this period would be absorbed by the green crops and returned to the soil at the beginning of a dry period.

SOIL ANALYSES ON WHEAT PLOTS, 1928-9.

Throughout the season 1928-9 soil samples were taken at frequent intervals from each of the 8 wheat plots in Stackyard field and the 5 wheat plots in Lansome field at depths of 0–6 in., 6–12 in. and 12–24 in., each sample being a composite one of 6–8 cores. With a minimum of delay the fresh soils were analysed for ammonia (in the first few samples by Matthew's aeration method and subsequently by the salt extraction method) and nitrate (by the phenoldisulphonic acid method). The salt treatments or extractions were always completed on the day of sampling.

The differences between the limed and unlimed series in Stackyard field and between the old and new series of plots in Lansome field were relatively small and the results for the corresponding pairs of plots have therefore been averaged in the following statements so as to reduce chance effects from soil irregularities in systematically arranged plots. The results are given in Fig. 1 for the first foot of soil only; Table I includes the averages for the second foot samples throughout the year. In Stackyard field it was possible to compare the permanent green manure-wheat plots with three other wheat plots under more usual rotations, viz. Series C, wheat in Norfolk rotation with either corn or cake fed to the sheep during the folding off of swedes, and a special strip of wheat immediately adjoining the mustard plots and following potatoes manured with dung. The average values for these three rotational wheat plots are also given in Table I.

During the winter and early spring the wheat after green manures stood out as by far the best and most forward wheat on the farm. Up to the beginning of May there was little difference between the Lansome and the Stackyard plots. This may be illustrated by the figures in Table I for the average nitrogen contents of the plants on May 9 (2·1 mg. and 2·5 mg. for Stackyard and Lansome).

From this time onwards the Stackyard wheat made very slow progress but the Lansome wheat grew vigorously. The difference between the two fields is well illustrated by the average nitrogen contents per plant on June 16 (4·3 mg. in Stackyard and 10·5 mg. in Lansome). In the five weeks' interval the nitrogen content of the Stackyard wheat had doubled, but that of the Lansome wheat had increased fourfold. This difference became steadily greater right up to harvest.

Fig. 1 also shows the monthly rainfall. A very wet October and a normally wet November were followed by an exceptionally dry and cold

spell in January, February, and March with a pronounced drought again in June, July, and August.

Table I.

	Stackyard field. Green manures eaten off Wheat after			Lansome field. Green manures ploughed in Wheat after		
	Tares	Mus-tard	(Rota-tion)	Tares	Mus-tard	(Fal-low)
Ammonia N in p.p.m. soil 0–12 in.						
Means of three dates of sampling						
Nov.–Dec. 1928	8·6*	9·4*	5·9*	—	—	—
Jan.–Feb. 1929	7·5†	5·3†	5·4†	7·8	5·7	—
Mar.–Apr. 1929	2·5	4·6	2·8	7·5	5·1	7·5
May–June 1929	1·2	1·1	1·2	1·8	2·3	2·3
Mean of all determinations: 0–12 in.	3·5	3·9	3·0	5·1	4·0	(4·2)
12–24 in.	3·3	3·8	2·9	3·6	5·1	—
Nitrate N in p.p.m. soil 0–12 in.						
Means of three dates of sampling						
Nov.–Dec. 1928	1·8	1·6	2·9	1·6	0·9	0·9
Jan.–Feb. 1929	0·9†	0·6†	1·0†	1·0	0·9	1·2
Mar.–Apr. 1929	1·7	0·9	1·4	2·0	1·7	1·8
May–June 1929	1·1	1·0	1·4	1·6	1·6	1·4
Mean of all determinations: 0–12 in.	1·3	1·0	1·3	1·6	1·2	1·3
12–24 in.	1·0	0·9	1·6	1·4	1·1	1·1
Mean N content per plant in mg. on May 9, 1929	2·2	1·9	3·7	2·7	2·3	3·0
Mean N content per plant in mg. on June 16, 1929	3·8	4·7	8·2	11·3	9·6	7·3
Yield of wheat in bushels	7·1	6·7	14·9	34·5	26·7	18·1
Yield of straw in cwt.	7·7	6·5	—	29·7	21·5	23·9

* 1 sampling only.   † 2 samplings only.

The outstanding results of the soil analyses are the very low nitrate contents throughout the year and the relatively high ammonia contents in winter and early spring. It is quite counter to earlier experience to find consistently more ammonia than nitrate in a soil, but it appears that few determinations of ammonia have been made during cold winters. It appears that the autumn rains washed out any nitrate formed during the three months fallow on Stackyard or immediately after the ploughing in of green manures on Lansome, and that subsequently the temperature was too low for nitrification. It is to be expected that there would be less readily nitrifiable material in Stackyard than in the Lansome plots in which the green manures were ploughed in quite shortly before the wheat was sown. There is evidence in the Lansome plots but not in the Stackyard ones of slight nitrate accumulation during April and May before the period of active growth of wheat. Although the differences were small, the Stackyard tares plots gave quite consistently higher

nitrate values than the mustard plots. The ammonia contents in both
fields show progressive falls from December to June (from about 8 to 1
parts of N per million). The ammonia contents remained high much longer
in Lansome field than in Stackyard. There is evidence in Stackyard field of
an increased availability of nitrogen from mustard during early summer.
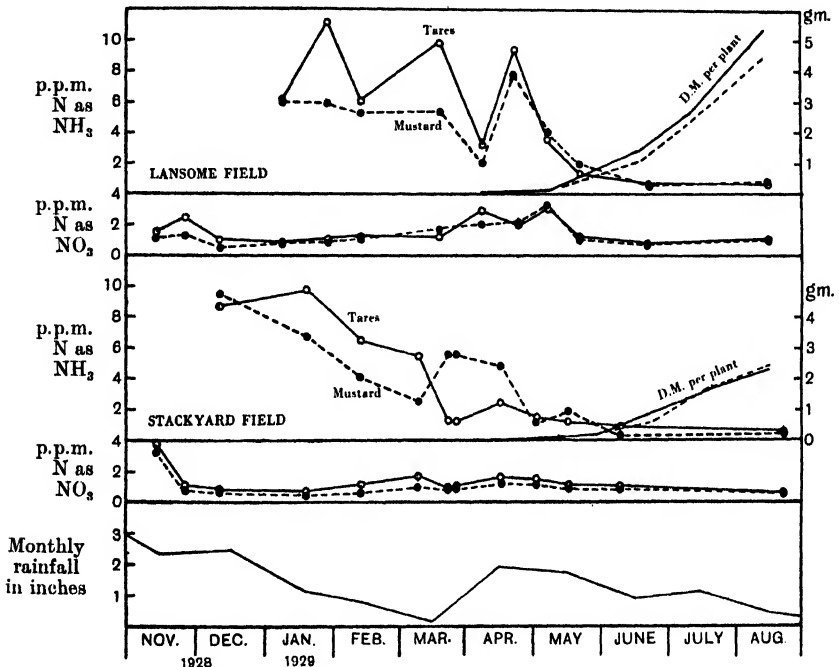
Fig. 1. Changes in ammonia and nitrate contents of soils during the growth of wheat
    after mustard and tares with data for the size of wheat plants and the monthly
    rainfalls. Woburn Stackyard field and Lansome field plots, 1928–9.

The data demonstrate a most acute nitrogen deficiency throughout
the year, but the nitrate contents do not differ sufficiently to account for
the wide differences in yield between the two fields. It must be remem-
bered however that under such conditions as these, nitrate contents can
give no measure of soil fertility. The nitrate measured represents the
balance between production and loss by assimilation by plants and lower
organisms and loss by leaching. On this light sandy soil it is clear that
these losses are so high that there is no opportunity for the actual ac-
cumulation of nitrate in amounts at all comparable with those obtained
in most of the published data for seasonal fluctuations in arable soils.
The extra nitrogen in the Lansome field must have remained locked up

in an organic form throughout the winter and that liberated in early summer must have been taken up immediately by the wheat.

GREEN MANURE PLOTS, 1929.

Similar analyses were made in 1929–30 on the other half of Stackyard field starting with the first green manure crops in 1929. The field had been subjected to vigorous cultivation operations before the mustard and tares were sown (May 30). Owing to the dry summer the yield of mustard
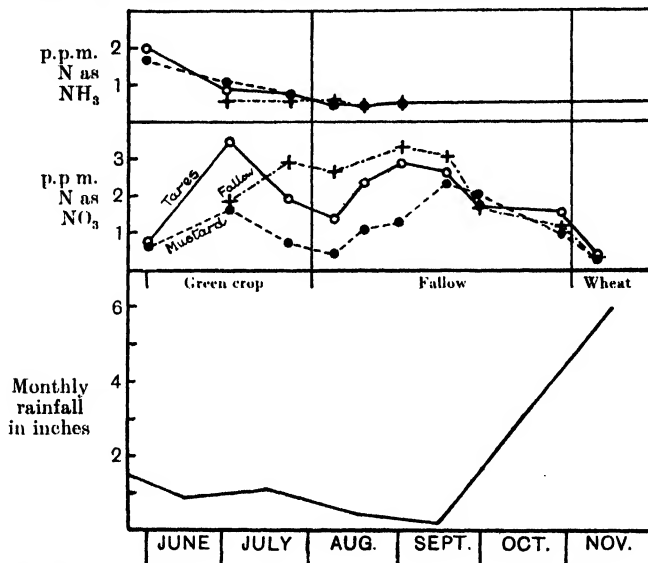
Fig. 2. Changes in ammonia and nitrate contents of soils during the growth of tares and mustard followed by fallow and wheat, with the monthly rainfalls. Woburn Stackyard field plots, 1929.

was low (2300 lb. fresh weight per acre) and that of tares was very poor (460 lb. fresh weight per acre). The crops were consumed on the land by sheep with the usual supplementary food of 3 cwt. of cotton cake per acre (July 30–August 1). The continued drought made it improbable that successful second crops could be grown and the plots were therefore fallowed with frequent harrowings. Wheat was sown early in November.

Fig. 2 gives the rainfall, ammonia, and nitrate contents for the unlimed tares and mustard plots and for an adjacent fallow plot. At the beginning of June the soil had again more ammonia than nitrate, but the ammonia contents fell off regularly and never reached amounts comparable with those of the previous year (mean value for winter 1929–30, 1·0 part N as NH_3 per million soil).

At the end of July the fallow strip had the highest nitrate content and the mustard plot had much less nitrate than the tares plot, as would be expected by its greater bulk and its utilisation of soil nitrogen. During the continued drought of August and September the nitrates remained almost constant in the fallow plot. In the tares plot they rose rapidly, and in the mustard plot more slowly, to give about equal values for all three plots by the end of September, when the drought broke. In all plots the nitrate content was rapidly reduced to about 1 part of nitric N per million of soil, at which value it remained throughout the year.

The second series of soil analyses adds further evidence for acute nitrogen deficiency in the wheat after green manures.

FIELD EXPERIMENTS 1929 AND 1930.

Direct tests for nitrogen deficiency were made in 1929 and 1930 on wheat after green manures in Stackyard field by very small scale experiments on top dressings with nitrate of soda. To secure the best comparison of the mustard and tares plots possible in absence of replication of the green manuring plots, the microplots were arranged in long narrow strips on either side of the boundary between the tares and mustard. In 1929 the produce of each plot was harvested and threshed separately and composite samples analysed. In 1930 the wheat was damaged by straying sheep in July, and the total produce was weighed without threshing. A selection from the developmental data and the final yields are given in Table II.

In both years the early applications had an immediate and striking effect in improving the colour, vigour, and tillering. Unfortunately the shoot counts in 1929 were not commenced until the maximum was passed, but the later counts show that whilst the early dressing had greatly increased the shoot numbers, the second dressing was too late to increase the number or the height of the ears, but it had greatly increased grain formation, as shown by the total yield and by the weight per ear. The essential difference between the ways in which early and late dressings act is well shown by the nitrogen percentage of the grain. Early nitrogen had no effect, showing that the nitrogen was utilised efficiently for carbohydrate synthesis and growth; late nitrogen increased the nitrogen percentage. Ear weight and nitrogen percentage in grain were closely correlated. The differences between the tares and mustard plots are relatively small in the absence of late nitrogen. Wheat after tares made less use of the late nitrogen, especially where early nitrogen had been

Table II. *Microplot experiments on Wheat after green manures.*

1928–9.

O = no manure.
E = 1 cwt. NaNo₃ per acre on April 16, 1929.
L = 1 cwt. NaNO₃ per acre on June 6, 1929.
E + L = 1 cwt. NaNO₃ per acre on April 16 and June 6, 1929.

For each green manure plot 4 × 4 plots each 0·00135 acre.

	After tares		After mustard	
	O	E	O	E
Shoots per metre May 14	54	64	51	71
Shoots per metre May 29	49	63	40	61

	After tares				After mustard			
	O	L	E	E + L	O	L	E	E + L
Shoots per metre June 24	36	34	43	42	46	44	46	55
Shoot height in cm. June 24	35	36	48	43	32	33	42	38
Shoot height in cm. July 18	83	82	88	85	72	77	83	87
Grain in cwt. per acre*	6·6	7·9	8·9	9·2	6·0	8·7	8·9	11·3
Straw in cwt. per acre	8·7	11·2	12·8	12·8	9·9	11·6	12·8	13·6
Ear weight in gm.	1·43	1·62	1·43	1·59	1·12	1·48	1·37	1·70
N per cent. of grain	1·34	1·56	1·32	1·56	1·27	1·47	1·22	1·52
N per cent. of straw	0·49	0·45	0·38	0·43	0·38	0·48	0·34	0·38
Percentage recovery of added N	—	27	22	21	—	41	23	34

* Standard error: after tares 0·56 (= 6·9 %); after mustard 0·75 (= 8·6 %).

1929–30.

O = no manure.
1 E, 2 E = 1 cwt. or 2 cwt. NaNO₃ per acre on March 8, 1930.
1 L, 2 L = 1 cwt. or 2 cwt. NaNO₃ per acre on May 23, 1930.
1 E + 1 L, 2 E + 2 L = 1 cwt. or 2 cwt. NaNO₃ per acre on March 8 and May 23, 1930.

For each green manure plot 3 × 8 plots each 0·00175 acre.

	Maximum shoot number per metre		
	O	1 E	2 E
After tares	63	76	86
After mustard	82	90	99

Yield of total produce in cwt. per acre.

	After tares					After mustard		
	O	1 E	2 E			O	1 E	2 E
O	10·2	17·2	20·6		O	14·6	20·5	24·9
1 L	18·0	19·8	—		1 L	21·3	26·1	—
2 L	20·7	—	29·6		2 L	22·4	—	31·5

Summary. Yields of total produce in cwt. of wheat grain + straw per acre (average of tares + mustard plots).

	1929				1930		
	O	1 E			O	1 E	2 E
O	15·6	21·7		O	12·4	18·9	22·8
1 L	19·7	23·5		1 L	19·7	23·0	—
				2 L	21·6	—	30·1

given. The assimilation of the added nitrogen was very great for late applications to wheat after mustard.

In the 1930 experiment the effects on tillering are represented in Table II by the mean maximum shoot number. The general results are similar to those of 1929. Indeed for the four treatments common to the two years the wheat after mustard yields agree to about 1 cwt. per acre. The wheat after tares gave much lower yields in 1930 than in 1929.
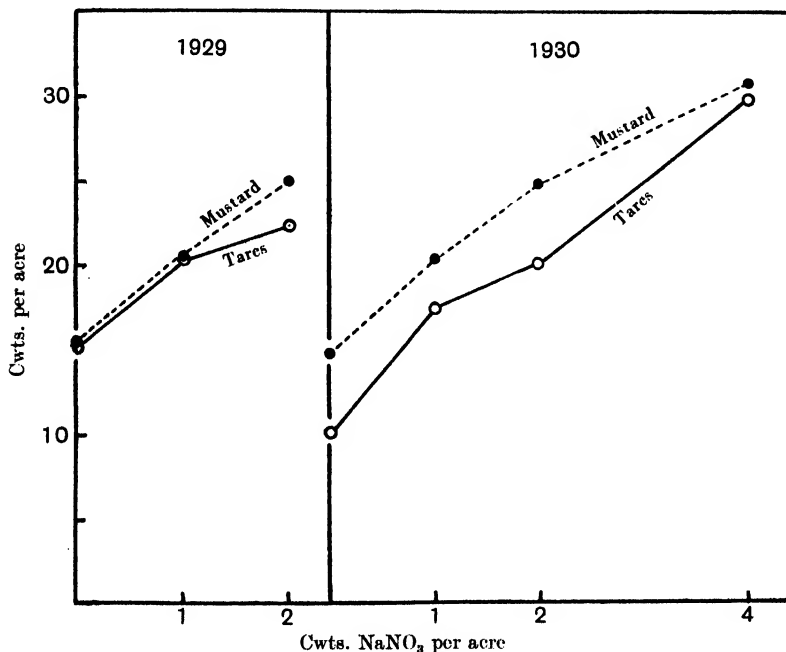
Fig. 3. Yields of grain and straw of wheat after tares and after mustard as functions of the amount of sodium nitrate given in top dressings. Woburn Stackyard field plots, 1929 and 1930.

Again the wheat after tares made little use of the single late dressing when an earlier one had been given, but it utilised the repeated double dressing as effectively as wheat after mustard.

The summary in Table II for total produce taking tares and mustard together, and that in Fig. 3 for the two crops separately, show that over a wide range the yield approximates to a linear function of the amount of nitrogen added. Although the maximum crop was still but a moderate one, there can be no doubt that, whatever the mechanism, tares and mustard fail to give a good preparation for wheat because they are

unable to provide available nitrogen in early summer at the most important period for the growth of wheat.

SUMMARY.

1. The Woburn field experiments on wheat after green manures are briefly reviewed. Contrary to the original expectations the wheat was less good after two summer crops of tares than after two mustard crops. This result was obtained many times and in recent years the wheat yields were extremely low after both green manures.

2. Regular soil analyses for nitrate and ammonia through 1928 and 1929 showed that the mean nitrate content was extremely low (1·2 parts of nitric nitrogen per million of soil). During the cold dry winter of 1928-9 the ammonia nitrogen was several times greater than the nitrate nitrogen.

3. Further evidence of an acute nitrogen deficiency during the critical period for the wheat plant in May and June was afforded by the large responses to top dressings of sodium nitrate both in the 1929 and the 1930 wheat crop.

4. The view is advanced that tares and mustard as summer crops fail to give a good preparation for wheat because they are unable to provide available nitrogen in early summer at the most important period for the growth of wheat.

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INVESTIGATIONS INTO THE INTENSIVE SYSTEM OF GRASSLAND MANAGEMENT.

By the Agricultural Research Staff of Imperial Chemical Industries, Limited.

VII. THE DIGESTIBILITY AND FEEDING VALUE OF GRASS SILAGE MADE IN A PIT.

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INTRODUCTION.

THE digestibility of grass silage made in a tower silo and in a stack has been dealt with in two previous papers in this series (1, 2). The making of silage in a pit is common practice where no silo has been erected, and this method is often advocated in preference to the stack. The sample to be described was made under conditions unfavourable to the production of first-class silage and was the result of an attempt to preserve a crop of grass destined for hay but which could not be made owing to unsuitable weather conditions, the grass having grown beyond the optimum stage for making hay.

MATERIAL AND METHOD OF TREATMENT.

In 1930 the growth of grass was very marked in the early part of the season and a large surplus was available in June. The grass was obtained from an area which had been intensively treated and received a dressing of phosphates, potash and nitrogen in March. The grass used for making the silage was not cut until June 23, by which date it had made considerable growth and had gone to the flowering stage.

The silage was made in a concrete pit 6 feet deep by 6 feet wide which had a concrete bottom with no provision for drainage. The grass was cut in the morning and was carted soon after. The pit, which was 12 feet long, was filled with grass which was well trampled down and was built up to a height of 6 feet above ground level.

When the silage had settled slightly, some blocks of concrete were laid on the surface to weight it down and the whole settled to ground level. Temperatures were taken in the middle of the silage during the period it was in the pit.

Table I. *Temperature of grass silage made in a pit.*

Date	Temperature (° F.)	Date	Temperature (° F.)
June 24	74	June 30	140
June 25	157	July 3	138
June 26	152	July 8	132
June 27	150	July 16	145
June 29	147		

These figures show that the temperature rose rapidly and reached a level of 157° F. within 48 hours, falling somewhat thereafter, though it remained at a fairly high level throughout.

The silage was used from July 8 onwards, and it will be noticed that cutting into the mass caused another rise in temperature.

There was considerable waste round the edges of the pit which showed that the grass had not been properly packed. This was due to two causes. The sides of the pit were vertical and the grass clung to the sides as the mass settled so that the silage was more compact in the centre of the pit. It was evident that the sides should have been given a slight slope, narrowing at the bottom. Such a formation would ensure tighter packing as the grass settled. Furthermore, the grass was somewhat stemmy and was not chaffed before it was filled in so that the texture of the whole mass was an open one.

The silage was cut out and fed to dairy cows, on grass, a fortnight after it was put into the pit. The silage was of a good colour and pleasant aroma and would be classed as sweet silage. It was eaten readily by the cows.

Composition of the silage.

The grass from which it was made was of poor quality when cut, as it had gone beyond the optimum stage for hay. This is reflected in the composition of the silage.

Table II. *Composition of pit silage made from grass.*

	% of original	% of dry matter
Moisture	74·92	—
Ether extract	0·53	2·10
Fibre	8·72	34·78
Crude protein	2·62	10·44
Ash	1·92	7·66
Nitrogen-free extractives	11·29	45·02
	100·00	100·00
True protein	1·87	7·46
Lime (CaO)	0·17	0·68
Phosphoric acid (P_2O_5)	0·18	0·74
Ratio: $\dfrac{\text{True protein}}{\text{Crude protein}}$ ...	0·71	0·71

The fibre content is high, whilst the figures for crude protein and ash are low.

Digestibility of the silage.

The digestibility was determined with two wether sheep by the usual method. A daily ration of 4 kg. of fresh silage was given per sheep. The silage was eaten readily and the uneaten residues were small, amounting to an average of 520 gm. of dry matter per sheep over the experimental period of 10 days. The trial was preceded by a pre-experimental period of 4 days' duration, commencing after the sheep had become used to a diet of silage alone.

The agreement between the two sheep was very satisfactory. The digestibility coefficients, calculated in the usual way, are given in Table III.

For comparative purposes, the values obtained in the trials, noted above (1, 2), with grass silage made in a tower and in a stack, are included together with values calculated from the figures given by Kellner (3) for the total and digestible constituents in grass silage made in a heap and in a pit.

Table III. *Digestibility coefficients of grass silage.*

	Pit silage (%)	Tower silage (1) (%)	Stack silage (2) Sample 1 (%)	Stack silage (2) Sample 2 (%)	Grass silage from heap (3) (%)	Grass silage from pit (3) (%)
Dry matter ...	60·0	71·7	60·9	61·1	*	*
Organic matter	60·7	73·0	63·4	63·4	57	59
Ether extract	62·0	77·7	64·1	69·5	48	50
Fibre	68·2	77·3	80·0	77·7	60	58
Nitrogen-free extractives	59·2	71·7	62·4	62·0	58	58
Crude protein	39·9	67·7	35·4	40·2	50	70
True protein...	24·8	49·5	24·9	28·7	*	*

* No figures available.

The digestibility of the pit silage is similar to that of the stack silage, though the fibre would appear to be somewhat less digestible. In this connection, however, it should be remembered that the crude fibre content was higher than would normally be the case with silage, which should preferably be made from grass before it has reached the flowering stage. The values quoted by Kellner are, in the main, of the same order as those obtained for the pit and stack silage made from grass at Jealott's Hill. The digestibility of the crude protein of the pit silage is lower than the value given by Kellner for silage made in a heap and considerably lower than that recorded by him for grass silage made in a pit. In this

connection it is interesting to note that the grass silage made in a tower is of a high order of digestibility throughout, this being particularly noticeable in the case of the protein.

If the unsuitability of the original material be taken into consideration, the silage made in a pit at Jealott's Hill is satisfactory and compares favourably with the values quoted by Kellner[3] for meadow hay.

Table IV. *Digestibility coefficients of pit silage,
medium and poor meadow hay (Kellner).*

	Meadow hay, medium	Range	Meadow hay, poor in protein	Range	Pit silage
	%	%	%	%	%
Organic matter	61	50–67	56	46–59	60·7
Crude protein	57	47–67	50	35–61	39·9
Ether extract	51	—	49	—	62·0
Nitrogen-free extractives	64	53–73	59	49–65	59·0
Fibre	59	50–71	55	46–64	68·2

The digestibility of the fibre and ether extract is higher in the pit silage but the digestibility of the crude protein is lower than either of the hays though it will be seen from the range of variation that in the latter case the digestibility coefficient of the crude protein in poor meadow hay may drop to as low a figure as 35 per cent.

A similar depression of the digestibility of the crude protein has been noted by Woodman[4] and is tentatively attributed by him to the effect of the high temperature attained by the material on which he was working, rye-grass and clover silage made in a stack.

The temperatures recorded in that work are similar to those reached in the present investigation, though the depression of the digestibility is not so marked in the pit silage.

The results of the digestibility trial show that the crop of grass has been conserved without undue loss, since it would have been impossible to make good hay at that particular time.

NITROGEN BALANCE.

The nitrogen balance shows that there has been a loss of nitrogen from the bodies of the two sheep, though not a serious one. Both sheep lost weight during the period of the trial (Sheep C 5 lb. and sheep D 2 lb.).

Table V. *Nitrogen balance of sheep.*

Average daily intake	15·58 gm.
Average daily excretion in faeces	9·36
Average daily excretion in urine	8·79
Average daily loss	2·57

Mineral balance.

The calcium and phosphorus balances show a retention in the case of the former and a slight loss in the case of the latter constituent.

Table VI. *Calcium and phosphorus balances.*

	Calcium (CaO) (gm.)	Phosphorus (P_2O_5) (gm.)
Average daily intake	6·34	6·90
Average daily excretion	4·70	7·02
Average daily balance	+ 1·64	– 0·12

It would seem that the silage had an adequate calcium content and did not fall far short of the requirements of the sheep for phosphorus at the plane of nutrition achieved by the ration.

Feeding value.

The digestible constituents in the pit silage are given in Table VII, together with the values quoted by Kellner[3] for grass silage made in a heap and in a pit and also for poor and good meadow hays. The production starch equivalent has been calculated by the well-known method of Kellner[3] applying the correction for fibre which he advocates for green fodder.

Table VII. *The digestible constituents in grass silage and meadow hay (based on dry matter).*

	Pit silage made from grass (this experiment)	Grass silage from pits (Kellner)	Grass silage from heaps (Kellner)	Poor meadow hay (Kellner)	Good meadow hay (Kellner)
Digestible crude protein ...	4·16	7·2	5·9	4·0	6·3
Digestible ether extract ...	1·30	2·1	4·1	0·6	1·2
Digestible nitrogen-free extractives	26·65	24·2	23·4	22·5	30·0
Digestible crude fibre ...	23·72	19·6	18·4	19·2	17·6
Digestible protein ...	1·85	4·6	2·2	2·9	4·4
Starch equivalent per 100 lb.	42·21	40·7	38·4	22·2	36·2

The pit silage compares favourably, as regards starch equivalent, with the other two silages and is of greater value, on a basis of dry matter content, than either of the hays. The digestible protein is lower than that of the silage made in a pit, quoted by Kellner, and the good meadow hay, but the pit silage made at Jealott's Hill is comparable in this respect to the grass silage made in a heap and the poor meadow hay.

The fairest comparison is with poor meadow hay, since the grass would normally have been made into hay and, owing to unsuitable weather conditions, could only have been made into poor hay.

Under the conditions prevailing, the grass was conserved in the best possible way. Had it been chaffed before it was filled into the pit it is possible that it would have packed more tightly and produced a better type of silage, from the point of view of its digestible protein content.

The silage was fed to dairy cows and was eaten readily and with relish, having the desired effect of providing a bulk of succulent forage when grass was rather scanty.

The different state and quality of the grass used in this experiment and in the experiments reported on previously ((1) and (2)) in making silage in a tower, stack and pit render any exact comparison of the relative merits of these processes impossible.

Such a comparison is, however, being made this year by investigating the feeding value of grass cut from the same area and made into silage by the three different methods.

SUMMARY.

Silage has been made satisfactorily in a pit from grass cut at the flowering stage when it was impossible to make hay. The digestibilities of all the constituents compare favourably with meadow hay with the exception of the protein which shows a depression.

The silage is richer in starch equivalent than meadow hay, if compared on a dry matter basis, and equal in digestible protein content to poor meadow hay though not to good meadow hay. It is therefore better to make grass silage when weather conditions are bad rather than attempt to make hay which, under such conditions, would be of poor quality.

The silage was used within four weeks of being made and was eaten readily by dairy cows on grass.

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APPENDIX I.

Digestibility coefficients of pit silage made from grass.

	Dry matter	Organic matter	Ether extract	Fibre	Nitrogen-free extractives	Crude protein	True protein
SHEEP C:							
	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)
Grass eaten ...	9260	8551	195	3221	4169	967	691
Faeces voided ...	3749	3397	71	1067	1706	597	513
Weight digested ...	5511	5154	124	2154	2463	370	178
% digested ...	59·53	60·27	63·59	66·88	59·07	38·26	25·76
SHEEP D:							
Grass eaten ...	9392	8674	197	3267	4229	981	701
Faeces voided ...	3705	3366	78	997	1724	574	534
Weight digested ...	5687	5308	119	2270	2505	407	167
% digested ...	60·53	61·19	60·39	69·49	59·24	41·49	23·82
Average digestibility coefficients	60·03	60·73	61·99	68·19	59·16	39·88	24·79

APPENDIX II.

Weight of sheep.

	Sheep C (lb.)	Sheep D (lb.)
8 vii. 30	122	127
24 vii. 30	117	125

THE USE OF SODIUM HYPOBROMITE FOR THE OXIDATION OF ORGANIC MATTER IN THE MECHANICAL ANALYSIS OF SOILS.

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THE pipette method of mechanical analysis requires the preparation of a completely dispersed soil suspension and G. W. Robinson has shown that with ammonia as the dispersing agent, even after the removal of calcium carbonate and exchangeable calcium, it is not possible to disperse certain soils rich in organic matter. He therefore proposed the removal of the greater part of this organic matter by a preliminary oxidation by hydrogen peroxide in boiling solution. His method proved so successful that it was adopted as the official method of the International Society of Soil Science in Conferences at Rothamsted in 1926, and in Washington in 1927. Difficulties were encountered later in several countries and at the Prague Conference of the First Commission of the International Society in 1929 it was decided to re-examine the whole question of pretreatment of soils for mechanical analysis.

Apart from readily dispersed soils in which a preliminary oxidation is probably unnecessary, the principal difficulties have been that hydrogen peroxide is an expensive and troublesome reagent in the tropics, and that in some soils the peroxide is decomposed so rapidly by catalysis by manganese dioxide that it is extremely tedious if not impossible to complete the oxidation of organic matter. L. B. Olmstead, L. T. Alexander and H. E. Middleton[1] have modified the method by conducting the hydrogen peroxide oxidation in the presence of acetic acid which destroys the manganese dioxide and allows the oxidation of organic matter to proceed.

The use of acid and the prolonged boiling are objected to by some workers, especially as the oxidation is accompanied by the solution of relatively large amounts of iron and aluminium, and it is not clear whether these come from the organic matter or from the decomposition of inorganic colloids.

To overcome both types of objection to the hydrogen peroxide method the use of cold solutions of sodium hypobromite was examined

and gave good results. Subsequently it was found that A. Atterberg[2] had also used this method but had not developed it. A solution of sodium hypobromite causes rapid oxidation of the soil organic matter in the cold and in the presence of manganese dioxide, whether naturally occurring in the soil or added in amounts up to one-quarter of the weight of soil. Some black African soils were decolorised in a few minutes, whereas they remained black for hours in boiling solutions of hydrogen peroxide. No appreciable amounts of inorganic matter were dissolved. The traces of aluminium were much smaller than those making up the so-called "Loss on solution" in the hydrogen peroxide method.

Subsequently it was found that by using sodium hypobromite it was possible to effect considerable simplification and saving of time in mechanical analysis by comparison with the official hydrogen peroxide method. No acid treatment is required and addition of ammonia as a deflocculating agent is unnecessary; shaking may be reduced from 24 hours to 30 minutes in a mechanical shaker or may even be restricted to a vigorous shaking by hand for most soils.

EXPERIMENTAL.

The method as finally developed and tested on a number of soils, some of which were difficult to disperse by older methods, is as follows:

10 gm. of air dry soil are treated with 200 c.c. of a freshly prepared solution of sodium hypobromite obtained by adding 2·5 c.c. of bromine to 100 c.c. of cold N NaOH. The bromine may be measured in a small cylinder containing a few c.c. of water or, more conveniently where many determinations are made, from a special bromine burette in which the mouth is closed by a ground-in glass cock. On shaking the bromine and sodium hydroxide solution for a few seconds the bromine dissolves and the solution is ready for immediate use: 100 c.c. are added to the soil and the mixture shaken occasionally. After about 2 hours a second lot of 100 c.c. of sodium hypobromite is added and the mixture is allowed to stand overnight. For average soils without large amounts of organic matter oxidation for 4 or 5 hours has proved sufficient.

After the oxidation sufficient dilute ammonia is added to destroy excess hypobromite which would otherwise attack the filter paper. The soil is collected on a hard filter paper (such as Whatman's No. 50) and washed with about 100 c.c. of (roughly) N NaCl and then with 0·1 N NaCl. For soils with much organic matter the filtrate sometimes has a dark colour. Washing with 0·1 N NaCl is continued until the filtrate is colourless. A final washing is made with about 50 c.c. of distilled water,

but this may be reduced or omitted if water dropping from the filter funnel becomes turbid. The soil is transferred to a long cylinder and diluted to 500 c.c.

After mechanical shaking for 30 minutes the clay fraction and the clay + silt fraction are removed and weighed in the usual pipette technique. Most of the supernatant liquid is poured off and the sediment washed through a 0·2 mm. sieve (or one with 100 meshes to the inch) into a tall 400 c.c. beaker. The sand on the sieve is washed with a stream of distilled water, collected, and weighed as the coarse sand fraction. The fine sand fraction is freed from silt and clay by repeated decantations. The removal of coarse sand after the shaking and the pipette samplings instead of before them, as in the official method, has the advantages of eliminating irregularities from rubbing the whole of the soil mass on the sieve and of giving much cleaner sand fractions. Discrepancies in the results obtained in different laboratories have been traced to the retention of varying amounts of clay and silt particles as aggregates in the coarse sand fraction obtained in the usual way. The use of a tall column of suspension in a narrow beaker increases the accuracy and reproducibility of the separation of silt from fine sand. In the sodium hypobromite method there is no necessity to determine the so-called loss on solution and no need to add a deflocculating agent. The sodium taken up from the sodium hypobromite is sufficient to ensure deflocculation, even for soils containing calcium carbonate. The method has also been applied successfully to soils containing gypsum. The solubility of gypsum in N NaCl is about four times as great as that in water; considerable amounts may therefore be removed by prolonging the washing with N NaCl and small amounts of gypsum in the final suspension have less flocculating effect on the sodium clay than on suspensions of ammonium clay.

COMPARISON OF METHODS.

The sodium hypobromite method was compared with the International A method (H_2O_2, HCl, NH_4OH treatments) and with Puri's method[3], in which the only pretreatment is with N NaCl followed by the addition of small amounts of NaOH to the final suspension. A 2 per cent. soil suspension was used throughout, even though this appears to be too high for accurate separation of clay in soils containing large amounts of clay and silt. A 20 c.c. pipette with a straight tip was employed.

Up to the present tests have been made on 25 soils from several countries, most of them chosen as difficult to analyse by one or other of the common methods.

All results are given as percentages of the original air dry soil and both the oven dry and the ignited weights of fractions are given. Although the former are generally preferred, the ignited weights are better for comparing the degrees of dispersion secured by different methods when varying amounts of organic matter may be left in the fractions or when the clays may differ in hydration (*e.g.* Na-clay and NH_4-clay). The clay and the silt fractions in methods omitting oxidation may contain considerable amounts of organic matter and an inefficient dispersion may be obscured.

Table I gives results by the three methods for the six samples used in the co-operative work of the First Commission of the International Society of Soil Science proposed after the Prague Conference 1929 and reported to the Leningrad Congress in 1930, together with those for three additional Sudan soils. Only in one of these soils (No. 6, Zagreb; loss on ignition 8·6 per cent.) is there any appreciable difference between the NaCl and the NaBrO method. It is unfortunate that all but one of the series of soils chosen for detailed work by the Commission should have had such low organic matter contents that oxidation was unnecessary. The NaBrO method gives higher results than the H_2O_2 method through the more effective dispersion of the sodium clay, especially in the Sudan soils.

Table II gives similar comparisons for five British soils with appreciable amounts of organic matter. Some form of oxidation is obviously essential for the analysis of such soils by the pipette method. Pretreatment with NaCl dispersed only from one-third to one-tenth of the clay and the undispersed clay was found not as silt but as fine sand. The dry clay fractions contained relatively large amounts of organic matter. The differences between the hydrogen peroxide and the sodium hypobromite methods were small. In all cases NaBrO gave the higher clay fractions, presumably on account of the greater dispersion of the sodium clay.

A fen soil with 37·7 per cent. loss on ignition was used in an extreme test of the NaBrO method (Table III). Oxidation by hydrogen peroxide was very protracted and required more than 400 c.c. of H_2O_2 spread over 2 days. With the normal amount of NaBrO the clay content was lower and the silt content higher than by the H_2O_2 method, but with twice the normal amount of bromine the results by the two methods agreed closely. The NaBrO method was, of course, much simpler and more convenient to carry out. In spite of the large organic matter content of this soil pretreatment without oxidation was sufficient to disperse almost all the

Table I.

Soil	Treatment	Water	Loss on ignition	Calcium carbonate	Loss on solution	Coarse sand Dried	Coarse sand Ignited	Fine sand Dried	Fine sand Ignited	Silt Dried	Silt Ignited	Clay Dried	Clay Ignited
1. Rendzina (I.S.S.S.)	H_2O_2	7·7	9·1	2·4	1·0	0·7	0·6	6·6	6·5	21·0	19·4	62·5	55·4
	NaOBr	7·7	9·1	2·4	—	0·6	0·6	7·9	7·4	23·2	22·0	66·1	57·6
	NaCl	7·7	9·1	2·4	—	1·0	0·9	7·5	7·2	24·2	22·0	63·1	54·5
2. Alluvium (I.S.S.S.)	H_2O_2	2·9	5·1	0·6	0·9	3·8	3·7	31·0	30·6	33·1	32·7	30·3	27·4
	NaOBr	2·9	5·1	0·6	—	4·6	4·5	31·3	30·9	33·9	32·8	31·3	28·6
	NaCl	2·9	5·1	0·6	—	4·6	4·4	30·7	30·3	33·3	32·8	31·5	27·4
3. Gezira (I.S.S.S.)	H_2O_2	8·5	8·2	4·6	1·4	3·9	3·2	13·5	13·3	18·6	16·8	55·5	47·5
	NaOBr	8·5	8·2	4·6	—	5·0	3·8	14·4	14·2	10·4	13·1	63·4	52·2
	NaCl	8·5	8·2	4·6	—	4·8	3·7	14·3	14·1	15·7	13·1	61·7	53·1
4. Kassala (I.S.S.S.)	H_2O_2	6·3	8·1	—	0·9	0·1	(0·1)	5·4	5·1	46·3	43·3	41·8	35·0
	NaOBr	6·3	8·1	—	—	0·2	(0·2)	5·1	4·8	40·0	37·4	50·5	43·8
	NaCl	6·3	8·1	—	—	0·2	(0·2)	5·1	4·5	40·4	36·4	52·4	45·8
5. Szik (I.S.S.S.)	H_2O_2	3·9	5·9	—	0·9	0·0	0·0	33·6	33·4	33·7	32·2	33·2	29·4
	NaOBr	3·9	5·9	—	—	0·4	0·3	32·4	32·2	32·5	32·0	34·8	30·5
	NaCl	3·9	5·9	—	—	0·5	0·3	31·9	31·6	35·5	33·9	36·6	30·5
6. Zagreb (I.S.S.S.)	H_2O_2	3·1	8·6	—	2·5	1·8	1·7	28·6	28·2	36·9	35·2	29·5	25·6
	NaOBr	3·1	8·6	—	—	2·8	2·5	28·0	27·6	36·9	35·0	31·4	27·0
	NaCl	3·1	8·6	—	—	2·9	2·4	28·2	27·4	44·2	39·9	25·9	21·6
7. Sudan 29957/43	H_2O_2	6·4	7·9	0·8	0·7	12·2	11·9	12·6	12·2	15·8	15·1	54·2	45·4
	NaOBr	6·4	7·9	0·8	—	13·9	13·5	12·5	12·1	11·6	11·6	59·7	50·5
	NaCl	6·4	7·9	0·8	—	13·0	12·5	12·5	12·1	12·5	10·0	58·0	50·8
8. Sudan 22345/50	H_2O_2	5·2	8·1	1·8	1·4	0·1	(0·1)	20·0	19·2	45·2	40·8	25·2	20·7
	NaOBr	5·2	8·1	1·8	—	0·3	(0·3)	18·9	18·1	43·2	37·2	35·0	30·7
	NaCl	5·2	8·1	1·8	—	0·4	(0·4)	20·4	19·5	39·4	35·8	35·7	30·3
9. Sudan 38724	H_2O_2	7·1	8·1	4·5	1·0	5·9	4·7	16·1	16·0	19·6	18·3	51·0	42·2
	NaOBr	7·1	8·1	4·5	—	8·4	6·2	16·6	16·5	12·8	13·1	57·9	49·3
	NaCl	7·1	8·1	4·5	—	7·1	5·4	17·0	16·8	16·2	13·2	57·7	50·0

Table II.

Soil	Treatment	Water	Loss on ignition	Calcium carbonate	Loss on solution	Coarse sand Dried	Coarse sand Ignited	Fine sand Dried	Fine sand Ignited	Silt Dried	Silt Ignited	Clay Dried	Clay Ignited
10. Wales A. 133	H₂O₂	2·9	11·1	—	1·8	12·0	11·7	20·5	20·1	25·7	24·5	32·0	28·5
	NaOBr	2·9	11·1	—	—	15·1	14·6	21·5	20·9	25·8	21·8	31·5	29·9
	NaCl	2·9	11·1	—	—	15·2	14·4	27·1	24·9	34·9	30·2	14·5	11·7
11. Wales C. 94	H₂O₂	3·9	15·1	—	2·6	17·2	16·6	19·4	19·1	28·2	28·0	18·5	15·7
	NaOBr	3·9	15·1	—	—	21·0	20·2	17·8	17·3	28·6	27·4	21·7	17·3
	NaCl	3·9	15·1	—	—	21·3	19·7	39·8	33·3	9·5	7·8	2·8	1·6
12. Wales G. 203	H₂O₂	3·5	19·4	—	2·8	13·4	11·1	35·3	33·1	19·3	17·0	16·0	13·3
	NaOBr	3·5	19·4	—	—	15·8	12·8	36·8	34·0	20·5	15·5	17·5	15·5
	NaCl	3·5	19·4	—	—	14·6	11·9	50·3	42·5	11·9	8·7	3·5	1·9
13. Paleozoic Silt Soil, Shropshire	H₂O₂	2·9	10·4	—	0·7	0·7	0·6	16·2	15·9	45·0	43·7	25·7	23·5
	NaOBr	2·9	10·4	—	—	4·3	4·0	15·6	15·2	46·5	45·0	27·6	23·8
	NaCl	2·9	10·4	—	—	3·2	2·8	27·0	24·4	45·7	41·2	10·2	8·0
14. Craibstone, Scotland	H₂O₂	1·8	7·6	—	1·4	43·2	42·9	27·7	27·6	11·0	10·7	9·0	7·5
	NaOBr	1·8	7·6	—	—	42·8	42·5	28·6	28·3	12·3	12·0	10·7	8·5
	NaCl	1·8	7·6	—	—	41·7	41·0	34·7	33·2	15·7	12·6	4·7	2·7

Table III.

Soil	Treatment	Water	Loss on ignition	Calcium carbonate	Loss on solution	Coarse sand Dried	Coarse sand Ignited	Fine sand Dried	Fine sand Ignited	Silt Dried	Silt Ignited	Clay Dried	Clay Ignited
15. Fen-soil	H₂O₂	21·6	37·7	1·2	3·5	0·0	0·0	5·6	5·2	16·0	12·6	27·3	18·7
	NaOBr*	21·6	37·7	1·2	—	4·0	1·4	9·7	6·7	21·4	15·5	24·5	15·8
	NaOBr†	21·6	37·7	1·2	—	2·1	1·1	6·5	5·8	16·4	13·5	27·8	19·2
	NaCl	21·6	37·7	1·2	—	4·5	1·2	10·0	6·2	25·6	17·1	33·3	16·3
16. Barnfield subsoil, Rothamsted	NaOBr‡	8·4	8·3	—	—	1·8	1·7	11·1	10·9	7·5	7·3	72·6	63·9
	NaOBr§	8·4	8·3	—	—	1·9	1·9	12·7	—	10·3	10·9	74·0	63·1
	NaOBr‖	8·4	8·3	—	—	2·0	1·9	12·4	—	11·1	10·5	70·7	61·1

* 5 c.c. bromine. † 10 c.c. bromine.

‡ No gypsum. § 1 % gypsum. ‖ 5 % gypsum.

clay, but oven dry weights of clay and silt fractions were misleading; one-half of the clay fraction was volatile.

Oxidation is required in some soils to secure efficient dispersion and in others to prevent the inclusion of indefinite amounts of organic colloids in the clay fraction. It may happen that these two opposite effects will cancel out within the limits of accuracy usually required and so account for some of the cases in which preliminary oxidation was not found necessary.

The data given above are sufficient to show that the organic matter content alone does not determine whether or not oxidation is necessary to secure complete dispersion of the clay. As is shown in Tables I and II, the NaCl method failed to give complete dispersion of clay in soils 6 and 14 (with losses on ignition of 8·6 and 7·6 per cent. respectively), but succeeded with six other soils in Table I with 8 to 9 per cent. loss on ignition and with the fen soil with 37·7 per cent. loss on ignition. In a paper presented to the Leningrad meeting of the First Commission of the International Society of Soil Science, E. M. Crowther and E. Troell [4] suggested that oxidation is necessary for complete dispersion of the clay when the ratio of organic colloids (or total colloids) to the inorganic colloids (*i.e.* clay fraction) is high. Since the loss on ignition of the total soil (corrected for carbonates) depends on both organic and inorganic colloids, and the air dry moisture content depends primarily on the clay content and both of these quantities are commonly determined in conjunction with mechanical analyses, it was suggested that their ratio might be used to indicate the necessity for including oxidation in the pretreatment for mechanical analyses. Where the ratio of loss on ignition to air dry moisture content was more than 2·5, oxidation proved essential for complete dispersion of the clay in all soils tested.

The NaBrO pretreatment provides a useful method for removing humic material from soils in the preparation of representative samples of the inorganic colloids and for other purposes in which it is desired to avoid heating or decomposition.

SUMMARY.

1. The use of freshly prepared solutions of sodium hypobromite instead of boiling hydrogen peroxide solutions in the pretreatment of soils for mechanical analysis by the pipette method has the following advantages:

(*a*) Soils containing manganese dioxide or large amounts of organic matter may be oxidised rapidly without heat, whereas oxidation of such

soils by hydrogen peroxide is a tedious operation requiring large amounts of reagent.

(*b*) Possible changes in the clay through heating and the dissolution of considerable amounts of sesquioxides are avoided.

(*c*) The reagents are cheaper and more stable, especially in the tropics.

(*d*) It makes possible further simplification in the technique of mechanical analysis.

2. A new and more convenient method of mechanical analysis is proposed in which acid treatment and the addition of a special deflocculating agent are unnecessary, the time of shaking is greatly reduced, and the separation of the coarser fractions is made more precise. Where the results differ from those by the hydrogen peroxide method they may be explained by the more effective dispersion of the sodium clay used in the proposed method.

3. It is shown that oxidation is required for complete dispersion in soils in which there is a high ratio of organic to inorganic colloids.

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STUDIES ON SOIL REACTION.

VII. AN ELECTRODIALYSIS APPARATUS FOR THE DETERMINATION OF REPLACEABLE BASES IN SOILS[1].

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(With Two Text-figures.)

ALTHOUGH electrodialysis was used in soil research by Cameron and Bell[1] over 25 years ago, it aroused little interest until the work of Gedroiz and Hissink demonstrated the theoretical and practical importance of a knowledge of replaceable base contents, especially of acid soils. In 1926 Mattson[2] used the ordinary type of three-compartment cell with parchment diaphragms for soils and showed that the amount of hydroxides collected in the kathode chamber approximated to the total content of replaceable bases. For many purposes a single value giving the total replaceable base content would be even more useful than the replaceable calcium content and the possibility of obtaining this value by simple titration instead of by a series of difficult analyses for small amounts of alkali metals and alkaline earths in a concentrated salt solution, directed attention to using electrodialysis for the routine examination of soils. The large three-compartment cell is too slow and the dialysates are too bulky for this purpose. Bradfield[3] introduced a notable simplification by making use of the fact that the anions associated with the replaceable kations are colloidal and therefore unable to pass through a membrane. He therefore devised a two-compartment cell with a long alundum thimble as soil container and membrane and inserted the anode directly into the soil suspension. After a series of only partially successful attempts (see p. 491) to use electrofiltration and so dispense with special membranes, Crowther and Basu[4] devised a modified form of Bradfield's apparatus which is much more convenient where analyses of many soils are required. The present paper describes further improvements in this apparatus and suggests modifications in the technique which give greater precision to the data obtained and, in particular, correct for the presence of water-soluble salts.

[1] This paper is abridged from a portion of a "Thesis approved for the Degree of Doctor of Philosophy in the University of London."

DESCRIPTION OF APPARATUS.

In electrodialysis both kations and water move towards the kathode and it is therefore sufficient to maintain a supply of water to the anode vessel and to collect and analyse the kathode liquid. Since the evaporation and analysis of large volumes of solution is inconvenient, the efficiency of the apparatus is determined not merely by the rapidity with which the bases are removed but also by the amount of water passing through the membrane. In Bradfield's apparatus a large alundum thimble is held vertically in a concentric cylindrical kathode and the large surface of the membrane immediately between the electrodes allows a rapid transport of water, whilst that of the kations is relatively slow because the soil tends to settle down below the electrodes. In the present apparatus the whole of the soil but none of the uncovered membrane lies immediately between the electrodes. The whole of the water must pass through the soil mass, and this ensures a high ratio of ionic transport to endosmotic flow. Table I shows that the present form of apparatus gives much smaller volumes of dialysate, smaller current strength (and therefore less heating) and sometimes a considerable saving in time as compared with Bradfield's apparatus.

Table I. *Comparison of Bradfield's* (A) *and the modified* (B) *apparatus.*

Soil	Apparatus	Total bases (mg. eq. %)	Volume of dialysate (c.c.)	Duration (hours)	Current strength (milliamps.)
Woburn Wheat, Plot 7	A	5·00	1455	7·5	250–200
	B	4·95	455	6·0	180–80
Park Grass, Plot 4–1 U	A	13·25	4200	16·0	300–250
	B	16·30	625	4·5	200–80
Park Grass, Plot 5–1	A	4·90	1030	5·0	380–260
	B	4·85	625	5·5	120–60

A unit of 6 cells is shown in Fig. 1, and the details of the cell in Fig. 2. The cell consists of an alundum thimble (R 84 dense, 5·5 cm. high and 3·5 cm. external diameter) placed on a stout perforated nickel kathode in a Pyrex glass vessel which has a side arm at about 3 cm. above the base. The anode is a perforated gold (or better platinum) disc 3·1 cm. in diameter and is mounted so as to be immediately over and parallel to the surface of the soil in the alundum thimble. A battery of 6 cells is mounted in recesses in the ebonite top of a stand which carries the necessary terminals and wiring for supplying D.C. current at 110 volts, through an adjustable rheostat of at least 500 ohms and an ammeter which may be introduced at will into the circuit of any one cell or of all the cells. Higher voltages may be used with advantage.

A constant water level is maintained in the anode chamber by means of a syphon which acts as a support for the anode and is connected through rubber tubing and a tap to a central constant level reservoir fed from a large bottle. By means of a wooden stand the set of syphons and electrodes is readily introduced or removed from the anode chambers. The end of the syphon is constricted and turned up so as to prevent the entry of the gas bubbles produced by electrolysis.
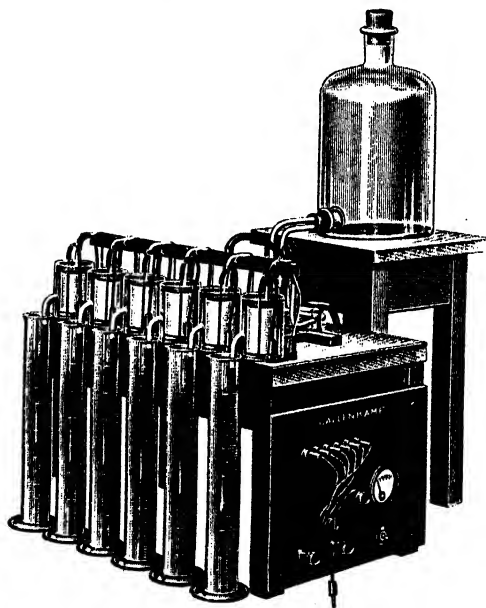
Fig. 1.
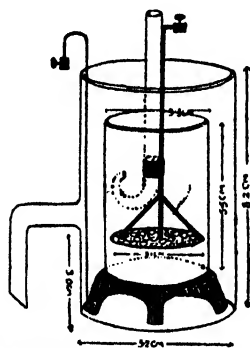
Details of Cells and Electrodes

Fig. 2.

The determinations are made on 10 gm. of soil passing a 1 mm. sieve. Smaller amounts should be used where the soils contain large amounts of replaceable bases; about 10 mg. eq. of replaceable base are most suitable. The dry soil is covered with a piece of filter paper, the kathode chamber is filled with water up to the side arm and the current is switched on immediately after introducing water into the anode chamber. In the early stages it is advisable to adjust the current to about 200 milliamps so that the soil is not heated to above 40° C. The dialysate is collected in successive lots of 250 c.c. (Certain modifications in technique are discussed later.) The rate of dialysis is generally about 200–300 c.c. per hour, and for most soils the process is substantially complete in about 6 hours. When the current falls to a low value the reaction of the solution in the

kathode vessel is tested with phenolphthalein and the dialysis should be continued for about half-an-hour after this ceases to the alkaline. A small amount of base, chiefly magnesia and calcium carbonate, adheres to the outside of the alundum thimble and to the kathode chamber and must be recovered. They are first washed in distilled water and the washings added to the dialysates which are boiled with a slight excess of $0.1\ N$ H_2SO_4. The alundum thimble is removed and, after cleaning all soil carefully from the inside, is placed in the boiling solution. When carbon dioxide is removed the cooled dialysate is passed through the kathode chamber to give a final washing and the excess acid titrated to the methyl red end-point in boiling solution. The solution may be used for the determination of calcium and magnesium. The total alkalinity of the dialysate gives the total replaceable base content, subject to certain errors discussed later which arise from the soluble salt content of the soil.

In some soils the thimbles may be used repeatedly, but in others they are stained with organic matter and sesquioxides and should be cleaned by diluted aqua regia followed by prolonged washing with boiling water. The final washing is always done by electrodialysis. After prolonged use and occasionally with some of a batch of new thimbles the rate of endosmotic flow may be found to be too low. This is remedied by soaking the thimbles overnight in $0.001\ N$ NaCl and, after drying, igniting them for several hours in a blast furnace. This appears to form an effective "glaze" on the membrane and to give it the requisite electrical charge. When not in use the electrodes must be kept dry.

COMPARISON OF ELECTRODIALYSIS AND AMMONIUM CHLORIDE METHODS.

Table II. *Woburn Wheat soils.* 0–9 *in.* *Replaceable bases in milligram equivalents per* 100 *gm. soil.*

By dialysis	Plot number									
	1	7	2A	2B	3B	4	5A	5B	6	11B
Ca	4·38	3·91	1·02	2·16	5·05	6·12	1·71	4·18	5·17	5·98
Total bases	5·62	5·10	1·99	2·63	6·00	7·27	2·96	4·63	6·89	6·99
Excess of results by NH₄Cl over those by electrodialysis										
Ca	0·18	0·17	0·15	0·06	0·09	0·10	0·11	0·11	0·07	0·08
Total bases	0·17	0·11	0·22	0·20	0·20	0·13	0·12	0·13	0·08	0·11

The results for differently manured plots on the Woburn acid sandy loam soil show good agreement between the electrodialysis and the usual ammonium chloride method, though the latter gives consistently higher

values (mean excesses 0·11 and 0·15 mg. eq. per cent. for Ca and total bases respectively). Determinations on separate lots of dialysate collected after 3, 6, 9 and 15 hours showed that all but mere traces (0·0 to 0·2 mg. eq. per cent.) of calcium was removed within 6 hours, but that magnesium continued to appear in the third and fourth dialysates. It seems unnecessary to prolong the determinations in the attempt to recover the whole of the magnesium, as the total amount is generally small.

Illustrations of the reproducibility of the results are given in Table III.

Table III. *Woburn Wheat soils (bases in mg. eq. per cent.).*

	Plot 1	Plot 2	Plot 3 B
Ca	4·58, 4·74, 4·63	3·91, 4·02, 3·87	5·05, 5·03, 5·05
Total bases (titration)	5·62, 6·10, 5·72	5·10, 5·20, 4·97	6·00, 5·95, 6·13

The advantage of the electrodialysis method over salt extraction methods is particularly marked for the light sandy soils hitherto discussed, as the analytical difficulties are very great by the older methods. The method has, however, been used successfully with heavier soils. Some difficulties were encountered with soils containing moderate amounts of water-soluble salts, and their examination led to proposals for further modifications in the technique of electrodialysis.

SOLUBLE SALTS AND ELECTRODIALYSIS.

In routine determinations by salt extraction methods it is customary to include the water-soluble salts in the replaceable bases. In some special determinations, as *e.g.* in R. Williams' [5] method for total bases by the ignition of the residue from an acetic acid extract, the true replaceable base content is obtained directly, as the soluble salts are not determined by the titration method adopted. In the electrodialysis results already quoted in this paper it has been assumed that bases equivalent to the kations of the soluble salts were included in the replaceable bases, and that the corresponding acids were retained in the anode chamber owing to the failure of the anions to pass through the negatively charged membrane under a high potential. The general agreement with the ammonium chloride results confirms this assumption, for the soils contain at least 0·5 mg. eq. of water-soluble calcium per 100 gm. soil, and the excesses of the ammonium chloride over the electrodialysis results are much smaller than this amount.

It was found, however, from certain deep subsoil samples from limed plots on Park Grass at Rothamsted that although the electrodialysis

results for calcium were reproducible (*e.g.* Plot 3 L, 18–24 in. gave 14·5, 14·4, 14·55 mg. eq. per cent.), those for total bases by titration of the dialysate were not only erratic but occasionally actually below the analytical results for calcium (*e.g.* 15·2, 16·2, 12·8, mg. eq. per cent. for same soil). Such soils had much more water-soluble calcium than the Woburn soils and the electroendosmotic transport of water was higher. It appeared that under these conditions the whole of the acid was not retained in the anode vessel; some escaped through the membrane into the dialysate and neutralised some of the hydroxides previously collected.

This difficulty was overcome by two modifications in technique which made it possible to determine at will either (1) the corrected values for the individual replaceable bases by analyses, or (2) the corrected values for the total replaceable bases by a single direct titration.

In the first method the salts are leached out of the soil before commencing the electrodialysis by setting up the apparatus as for a determination and running it overnight without applying the current. Owing to the high resistance of the leached soil, subsequent electrodialysis is slow, but it may be accelerated sufficiently by supplying a very dilute salt solution instead of distilled water to the anode vessel. Chlorides are undesirable, as the small amounts of chlorine liberated gradually attack the gold anodes. Ammonium sulphate was used in preference to sodium or potassium sulphate partly to make it possible to determine these metals by analyses and partly because it was found to cause less heating in the cell. With 0·001 N ammonium sulphate after leaching, the endosmotic flow proceeds more slowly (about 50 c.c. per hour) than with unleached soils even of low salt content, but the removal of bases proceeds at about the same rate. A further reduction is therefore effected in the volume of solution to be analysed. A small but indefinite amount of sulphuric acid is carried through the membrane, and it is not therefore possible to determine the total replaceable bases by direct titration. An analytical determination gives, however, the true replaceable calcium.

The second method gives by direct titration the total replaceable bases corrected for soluble salts. Unleached soil is electrodialysed against water, but precautions are taken to ensure that all of the soluble acids formed in the anode chamber shall pass through the membrane into the dialysate and neutralise the amount of base derived from the soluble salts. After each successive lot of 250 c.c. dialysate is collected (*i.e.* about hourly in the early stages), the electrodialysis is interrupted by closing the taps. The anode liquid is forced through the membrane by electro-

endosmose until the liquid falls below the anode and breaks the circuit. More water is added and this washing is repeated a few times. The taps are now opened and the normal electrodialysis continued. A direct titration gives the total replaceable bases without soluble salts, but the results of determination of individual bases include the amount present as salts.

Table IV. *Woburn Barley Plots, surface 0–9 in.*

All results in mg. equiv. per 100 gm. soil.

A. Replaceable Ca by N NH$_4$Cl method.
B. Water-soluble Ca.
C. Replaceable Ca by electrodialysis with 0·001 N (NH$_4$)$_2$SO$_4$ after leaching with water (true replaceable Ca).
D. Replaceable Ca by electrodialysis with the inclusions of anode liquid (true replaceable Ca + water soluble Ca).
E. Titratable bases by electrodialysis with the inclusion of anode liquid (true total replaceable bases).
F. Replaceable bases other than Ca by N NH$_4$Cl extraction.
G. ($=E$–C). Replaceable bases other than Ca, from combination of results of two methods of electrodialysis.
H. ($=D$–C). Water-soluble Ca, from combination of results of two methods of electrodialysis.
I. ($=A$–B). True replaceable Ca, by N NH$_4$Cl extraction with correction for water-soluble Ca.
J. ($=C$–I). Difference between true replaceable Ca by electrodialysis with 0·001 N (NH$_4$)$_2$SO$_4$ and by N NH$_4$Cl extraction.
K. ($=H$–B). Difference between soluble Ca by combination of two methods of electrodialysis and by extraction with water.
L. ($=G$–F). Difference between replaceable bases other than Ca by electrodialysis and by N NH$_4$Cl extraction.
M. ($=D$–A). Difference between replaceable Ca (including water-soluble Ca) by electrodialysis and by N NH$_4$Cl extraction.

Plot No.	A	B	C	D	E	F	G (E–C)	H (D–C)	I (A–B)	J (C–I)	K (H–B)	L (G–F)	M (D–A)
1	3·76	0·42	3·20	3·56	4·16	1·10	0·96	0·36	3·35	−0·15	−0·06	−0·14	−0·20
2 A	1·16	0·68	0·54	0·97	1·01	1·07	0·47	0·43	0·48	+0·06	−0·25	−0·60	−0·19
2 B	4·78	0·65	4·25	4·76	4·92	0·99	0·67	0·51	4·13	+0·12	−0·14	−0·32	−0·02
3 B	4·95	0·58	4·40	4·82	5·37	1·08	0·97	0·42	4·38	+0·02	−0·16	−0·11	−0·13
4 A	4·96	0·49	4·36	4·85	5·30	1·40	0·94	0·49	4·48	+0·00	−0·12	−0·46	−0·11
5 A	1·56	0·41	1·27	1·56	2·91	1·75	1·64	0·29	1·15	+0·12	−0·12	−0·11	−0·00
5 B	6·01	0·68	5·18	5·64	6·25	1·49	1·07	0·76	5·34	−0·16	−0·22	−0·42	−0·37
6	5·46	0·54	5·05	5·71	5·89	1·51	0·84	0·66	4·92	+0·13	+0·12	−0·67	+0·25
7	4·03	0·54	3·31	3·64	4·23	1·07	0·92	0·33	3·49	−0·16	−0·21	−0·15	−0·39
11 B	6·44	0·96	5·22	5·83	7·11	2·32	1·89	0·61	5·48	−0·26	−0·35	−0·57	−0·61

Table V. *Park Grass, Rothamsted Plot 3 L.*

Depth in in.	A	B	C	D	E	G (E–C)	H (D–C)	I (A–B)	J (C–I)	K (H–B)	M (D–A)
0–6	19·6	1·3	17·4	18·1	21·3	3·9	0·7	18·3	−0·9	−0·6	−1·5
6–12	13·9	1·3	12·3	13·8	15·2	2·8	1·5	12·6	−0·2	+0·2	−0·1
12–18	14·7	1·1	11·9	12·5	13·9	2·1	0·7	13·6	−1·7	−0·5	−2·2
18–24	15·6	5·6	9·1	14·5	11·8	2·7	5·4	10·1	−1·0	−0·2	−1·1
24–30	17·5	4·4	13·9	18·0	15·0	1·2	4·1	13·0	−0·8	−0·3	+0·5
30–36	16·3	3·1	12·6	14·4	12·8	0·2	1·8	13·2	−0·6	−1·2	−1·8

Typical results are given in Table IV for a sandy Woburn soil with low replaceable bases and salt contents and in Table V for a limed Rotham-sted clay loam soil in which the amount of water-soluble salt (up to 5·6 mg. eq. per cent.) exceeds the replaceable calcium of the Woburn soil. For the lighter soil the electrodialysis method agrees well with the corrected salt extraction method for true replaceable calcium but less well for the uncorrected replaceable calcium. The combination of the results from the two electrodialysis methods gives low results for water-soluble salts and for other bases. The irregularities are greater in the heavier soils presumably through the incomplete removal of magnesium by electrodialysis and the accumulation of errors in the summation of a series of difficult analyses by the ammonium chloride method.

Although the modifications in the technique proposed have not been fully explored, it appears probable that, by defining more precisely the actual origin of the bases determined, they should increase the value of the electrodialysis method for the routine analysis of soils. The direct determination of total replaceable bases by the second modification should provide a particularly useful routine method as the manipulations and analytical work are extremely simple.

ELECTROFILTRATION.

The apparatus just described arose out of the failure of simpler forms in which the soil was used without a special membrane. For some tropical soils of low organic matter contents, promising results were obtained by a simple apparatus in which the soil was supported on a filter paper with a copper or nickel gauze as kathode between the filter paper and a glass filter funnel. A disc anode was placed immediately over the soil surface and a constant head of water maintained above the soil. Electrolysis and filtration were rapid and the calcium contents of the filtrate agreed well with the replaceable calcium, but the total alkalinity was lower than the total replaceable bases. Similar results were also obtained for some English soils, but the method failed in soils with moderate amounts of organic matter, owing to the large amount of acid leached through. Sometimes this was more than sufficient to neutralise all of the hydroxides previously obtained in the filtrate. The determination of total bases by titration was thus impossible, and in addition it seemed doubtful whether the method could be reliable for analyses of the filtrate in view of the extensive decomposition of organic matter and the large production of acid. In another apparatus Jena sintered glass

crucibles were used as anode vessels with gauze kathodes held by moistened filter papers close to the lower sides of the sintered glass plates, but the filtration and electrolysis were generally too slow to be convenient.

SUMMARY.

1. A simple form of two-compartment electrodialysis cell is described for the determination of replaceable bases in sets of six soils at a time. The determination requires little attention and the total replaceable bases are obtained by direct titration.

2. The disturbing influence of water-soluble salts is examined and it is shown that the technique may be modified so as to exclude the kations of soluble salts from either the total bases as determined by direct titration of the dialysate or from the individual bases as determined by analysis.

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WINTER LEACHING AND THE MANURIAL VALUE OF GREEN MANURES AND CROP RESIDUES FOR WINTER WHEAT[1].

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(With Seven Text-figures.)

ALTHOUGH systems of cropping light arable land in humid regions almost invariably involve a rotation, there is little precise information on the extent or mechanism of the effect of crops other than leys on the subsequent ones in the rotation. Precise field experiments with different rotations over periods of years are difficult to conduct, and the greatest successes in practice have been obtained by very elastic systems designed primarily to ensure a supply of sheep food at all times of the year. The most complete series of field experiments on light soils in England was commenced in 1876 by the Royal Agricultural Society at the Woburn Experimental Farm in Bedfordshire. In addition to repeating the Rothamsted continuous wheat and barley experiments it was decided to examine the residual effects of different manurial and cropping schemes throughout simple rotations. In a comparison of rich cake-fed dung with poorer corn-fed dung in a Norfolk rotation it was found, contrary to the beliefs embodied in the general farm practice of the time, that the benefit from the extra nitrogen in the cake was limited to the barley crop immediately following. Still more unexpected results were obtained in later experiments on simpler rotations. Winter wheat after summer tares (vetches—*Vicia sativa*) ploughed in was no better than wheat after summer mustard (*Sinapis alba*) ploughed in and, as the experiment continued, the yields of wheat declined very rapidly on plots with a tares-wheat rotation and rather more slowly but ultimately just as badly on the plots with a mustard-wheat rotation. Liming still further reduced the wheat after tares; mineral manures had no effect; and similar results were obtained when the whole experiment was repeated with similar summer green crops folded off by sheep which received cake in addition to the green crops. At Rothamsted, on the other hand, tares gave better results than mustard as a preparation for

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[1] This paper is based on data presented by T. J. Mirchandani in a "Thesis approved for the Degree of Doctor of Philosophy in the University of London."

wheat. This divergence of results at the two experiment stations is typical of the conflict of experience of farmers on the relative merits of these green manures in preparation for winter cereals. An adequate explanation of the Woburn results should not only lead to the more efficient utilisation of these green-manure crops in practice but should throw some light on the more fundamental problems involved in rotations. Changes in cropping systems are being made increasingly frequently at the present time through changes in economic conditions, and many well-tried practices, such as the Norfolk rotation and its simpler modifications, must often be abandoned. Whatever the immediate results, no system can be successful if the general level of soil fertility is reduced by the unintentional inclusion of factors such as those responsible for the failure of the Woburn wheat after green manures.

Many hypotheses have been advanced from time to time to account for marked decline in fertility of the Woburn green-manuring plots, especially with tares, and some of them have already been tested in field and pot experiments with negative results. As far as we can ascertain, our own hypothesis that summer green manuring on such soil leads to an acute shortage of available nitrogen at the time of the wheat's greatest need for nitrogen has not been advanced before. The facts that relatively large amounts of nitrogen were known to be added to the soil immediately before the wheat was sown, and that the wheat started off well and for six or seven months was ahead of wheat grown in usual rotations, appear to have prevented the recognition of the nitrogen deficiency during May and June, although it has been repeatedly observed that at this time first the wheat after tares and then the wheat after mustard appear to fall behind other wheat in the same field. The results described in the preceding paper demonstrated the existence of this nitrogen deficiency by both systematic soil analyses and small scale top-dressing experiments in two seasons.

The present paper describes laboratory and pot-culture experiments on tares, mustard, and other organic materials which reproduce in part the field results and support the hypothesis that after summer green manures the lack of available nitrogen at the critical period for wheat is due to excessive losses of nitrate by leaching especially during winter. Earlier pot experiments at Woburn failed to show any inferiority of wheat after tares to wheat after mustard, but in these earlier experiments the essential factor of leaching was ignored.

In the experiments to be described one half of the pots was leached periodically from January to March and the other half was unleached.

To secure greater control of the experimental conditions the tares and mustard were not grown in the experimental pots but added in suitable amounts to uniform lots of soil.

In the stage of growth in which they are usually ploughed in or folded off in the field, tares is a soft, leafy and essentially immature plant, whereas mustard has much hard stalk; tares is rich in protein and mustard in cellulose and lignin-like products. These differences may be summarised conveniently by stating that tares has a much narrower carbon-nitrogen ratio than mustard (actually 13 : 1 and 26 : 1 respectively for the samples used in these experiments). It is well known that the oxidation of carbon compounds by micro-organisms requires a suitable supply of combined nitrogen for the production of micro-organic protoplasm. Unless the C : N ratio of plant and animal products is less than about 20 : 1 their decomposition in the soil proceeds relatively slowly unless some additional supply of combined nitrogen is available and the decomposition converts some of this available nitrogen into microbial protoplasm. Decomposition of materials with less than about 20C : 1N liberates ammonia and ultimately nitrate. On these grounds the residues of a crop of tares would be expected to decompose rapidly and liberate an appreciable fraction of its nitrogen as nitrate, whereas the residues of mustard would reduce the nitrate content of the soil for a considerable period. These differences would be still further emphasised where the crops are consumed by sheep, for the greater part of the tares is eaten, whereas much of the mustard remains as hard stalks which will be less intimately mixed with the soil mass and therefore decompose more slowly.

The main purpose of the experiment was therefore to test whether this elementary distinction between tares and mustard is sufficient to account for the differences observed in the field or whether there are more specific effects, such as a partial sterilisation from the mustard oils or a toxicity of tares, as has sometimes been suggested. The materials added to the soil were adjusted to supply the same amount of nitrogen (6 mg. per 100 gm. soil). The elementary composition of tares was imitated by a mixture of mustard and a protein and that of mustard by a mixture of tares and a source of cellulose, and both C : N ratios were also provided by mixtures of protein and cellulosic materials without green manures. Partly to utilise constituents of ordinary manures and partly to avoid exaggerated leaching effects from soluble or very readily oxidisable substances, it was decided to use blood and straw for the mixtures.

The results of two series of nitrification experiments in the laboratory and of a series of pot experiments are presented separately.

<div align="center">THE MATERIALS.</div>

All of the plant products were air dried and finely ground in a disintegrator mill. This method of preparation may have caused changes in the compounds present in the plant products, but accurate sampling, intimate incorporation of the materials with the soil, and storage for subsequent experiments were more important than an exact reproduction of the conditions in the field on a certain date. The tares and mustard used in the major experiments were taken from the permanent green-manuring rotation plots on Stackyard field at Woburn at the end of July 1929 at the stage in which they were considered suitable for folding with sheep. In one of the laboratory experiments a sample of much younger mustard was used in addition. The farmyard manure was taken from the middle of a well-rotted heap of mixed pig and horse manure. The blood unfortunately proved to be a poor sample of low nitrogen content; the presence of some non-protein material, probably fat, may account for the relatively slow nitrification in all of the experiments. Table I gives the ultimate composition of the materials and Table II their rates of application to the soil throughout all of the

<div align="center">Table I. Composition of organic materials used.</div>

	% N	% C	C : N
Dried blood	11·10	41·5	3·7
Young mustard	3·48	34·7	9·9
Tares	3·01	40·1	13·3
Farmyard manure	2·15	30·9	14·4
Mustard	1·51	39·9	26·4
Straw	0·32	40·9	127·8

<div align="center">Table II. Mixtures supplying 6 mg. of nitrogen per 100 gm. of soil.</div>

C : N	Symbol		Organic manure	Blood	Straw	Organic manure	Blood	Straw
			Organic matter in gm. as			Nitrogen in mg. from		
13 : 1	*T*	Tares	0·200	—	—	6·00	—	—
13 : 1	*MB (T)*	Mustard + blood	0·170	0·031	—	2·56	3·44	—
13 : 1	*SB (T)*	Straw + blood	—	0·050	0·148	—	5·53	0·47
13 : 1	*F (T)*	Farmyard manure + blood	0·256	0·004	—	5·50	0·49	—
26 : 1	*M*	Mustard	0·400	—	—	6·00	—	—
26 : 1	*TS (M)*	Tares + straw	0·177	—	0·213	5·32	—	0·68
26 : 1	*SB (M)*	Straw + blood	—	0·044	0·344	—	4·90	1·10
26 : 1	*F (M)*	Farmyard manure + straw	0·248	—	0·206	5·33	—	0·66

experiments. These are of the same order as those supplied by the average field crops, but are much below those commonly used in ammonification or nitrification tests in the laboratory.

The soil for all of the laboratory experiments and for the major series of pot cultures was taken from a fallow plot between the green-manure plots and the continuous barley plots in Stackyard field, Woburn Experimental Station, and had been uncropped and unmanured for many years.

<center>THE LABORATORY EXPERIMENTS.</center>

It has often been demonstrated in these laboratories that the course of the microbiological activities in bottled soils is profoundly modified by variations in the degree of aeration, and that some approach to field conditions is obtained when the soil is contained in relatively large flasks and is shaken frequently to promote free gas exchange at the outside of the individual soil crumbs. Instead of taking a series of samples from a large mass of soil in a single flask a large number of separate 250 c.c. flasks containing 125 gm. of moist soil was set up so as to provide one for each analysis. In this way sampling errors were distributed at random throughout the experiment, and there was no concealed systematic change due to alterations in the degree of aeration throughout the experiment. The moisture content was adjusted to and maintained at 13·5 per cent. by frequent additions to constant weight. The flasks were plugged with cotton-wool and kept in a basement room with little daily temperature fluctuation. They were uncovered and shaken vigorously for one minute every day to ensure aeration.

Ammonia and nitrate were determined by Carsten Olsen's method [2]. 100 gm. of soil were shaken for one hour with 200 c.c. of N KCl containing enough HCl to give a pH value of about 1·0. The first 25 c.c. of the filtrate were discarded and 100 c.c. of the remainder were diluted and distilled with 3 gm. of magnesia into 0·02 N acid and the residue again diluted and distilled with 2·5 gm. of finely powdered Devarda's alloy.

To economise space only the nitrate results are presented. With tares and other rapidly decomposing materials the ammonium nitrogen reached about 2 mg. per 100 gm. soil after 7 days and then fell to 0·1 to 0·3 mg. per 100 gm. soil after about one month. The other materials had lower initial ammonium contents and the later ones were of the same order as with tares.

The first experiment was continued for 8 months from autumn to spring and the second for 4 summer months. Figs. 1 A and 1 B show

the nitrate contents of the soils receiving single substances in the two experiments. The smoothness of the curves shows that there were no great irregularities among the flasks, and that nitrate accumulation proceeded smoothly after the first month. The rates were naturally much more rapid in the summer period and the time scale has therefore been doubled in the figure.
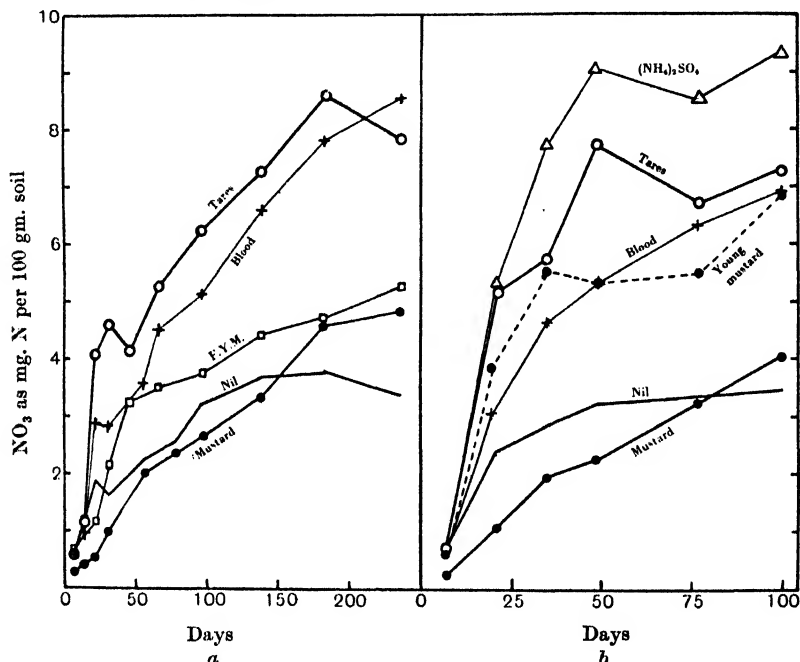
Fig. 1. Nitrate accumulation in soils receiving 6 mg. of organic nitrogen per 100 gm. soil. A, winter experiment; B, summer experiment (with expanded time scale).

ACCUMULATION OF NITRATE IN FLASKS.

The outstanding results are the rapidity and the extent of the nitrate accumulation from tares. The excess of nitrate over the untreated soil on the average of the last two points of the curves corresponds to 76 per cent. of the added nitrogen in the first and 62 per cent. in the second experiment. For nearly 6 months in the first experiment and throughout the whole of the second experiment tares gave more nitrate than blood, which is generally regarded as being very readily nitrifiable. In the second experiment the nitrification of tares nitrogen proceeded almost as rapidly though naturally less completely than that of ammonium sulphate. It will also be seen in Fig. 1 B that the sample of

young mustard ($C : N = 10 : 1$) nitrified about as rapidly as blood. Farmyard manure (Fig. 1 A) gave a steady but relatively small nitrate accumulation above that of the untreated soil after the first month. Mustard in the blooming stage, as used in the field experiments and in the pot experiments described later in this paper, immediately reduced the nitrate accumulation below that of the untreated soil and this effect lasted for some 5 months in the winter experiment and for at least 3 months in the summer one. The nitrification process was thus complete for tares before the decomposition of mustard had reached the stage at which it ceased to cause a drain on the nitrate and ammonia already present in the soil.

The results for the other mixtures used in the first experiment are set out in a condensed form in Fig. 2, which gives the means obtained by taking the 10 successive determinations together in groups of 1–3, 4–6, 7–8 and 9–10.

Each of the four sections of Fig. 2 shows the results for a pair of single substances or mixtures with $C : N$ ratios of 13 : 1 and 26 : 1 (those for the untreated soil are added as a base line). If the $C : N$ ratio were an adequate index of the availability of the nitrogen in organic manures the four sets of curves should be similar. Although the differences are always in the same direction, none of the other pairs shows as wide a divergence as that between the tares and mustard. With straw (Fig. 2 C) and with farmyard manure (Fig. 2 D) the differences due to $C : N$ ratio are about one-third to one-half of those between tares and mustard. In Fig. 2 B the close similarity between the tares + straw ($26C : 1N$) and the mustard + blood ($13C : 1N$) mixtures, and the fact that the two curves run just about midway between those for tares and mustard alone, might suggest either that a specific stimulating effect of tares just balanced a specific depressing effect of mustard or, what is much more likely, that an effect due to the chemical composition or physical condition of the materials works in opposite directions at high and low $C : N$ ratios. The essential difference between the single green manures and the mixtures is that in the former the proteins and carbohydrates occur in intimate association as relatively soft material readily open to micro-organic activity, whereas in the mixtures the proteins of the dried blood and the cellulose and lignin materials of straw occur in harder materials which are more slowly attacked and are necessarily at some distance from the particles of mustard and the tares added with them. If the decomposition of organic materials in the soil occurs as the result of associated activities amounting almost to symbiosis between a number

of groups of organisms, some liberating and others accumulating simple forms of nitrogen, it would be natural to expect this action to proceed more smoothly and rapidly where the sources of carbon and nitrogen occur in most intimate association. It was noticeable during the first month that the mixtures gave much more irregular curves than the
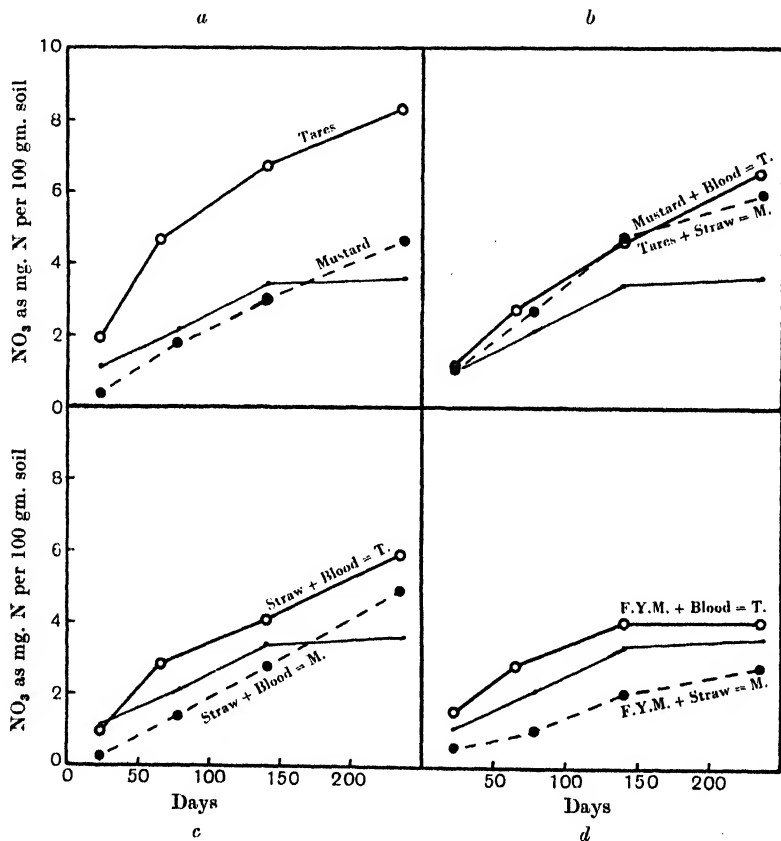
Fig. 2. Nitrate accumulation in soils receiving 6 mg. of organic nitrogen per 100 gm. soil with 13C : 1N (=tares) and 26C : 1N (=mustard) in comparison with untreated soil.

tares or mustard alone. This may be illustrated by the following figures for the variance of the three differences between successive pairs of the first four determinations of ammonia plus nitrate nitrogen:

Tares 0·18; equivalent mixtures 0·88, 0·37, 0·72.
Mustard 0·01; equivalent mixtures 0·67, 0·16, 0·72.

The greater variation between the results for the mixtures suggests that in the early stages, at any rate, there is a tendency for either the production or the consumption of inorganic nitrogen to'predominate at any one time or place and that the soil mass settles down to a steady state only very slowly. This may be illustrated too by another form of expressing of the results of the second experiment, which included, in addition to the series presented in Fig. 1 B, determinations on samples of tares, mustard, and young mustard, which had been extracted with a normal solution of potassium sulphate to remove the more readily salt-soluble proteins and carbohydrates, and also series in which these extracted materials were accompanied by quite small amounts of blood. (It may be mentioned that the extracted old mustard had a much larger C : N ratio than the original material and removed almost all the nitrate; extraction of the tares and young mustard had little effect on their C : N ratios and slightly reduced the accumulation of nitrate.)

The nitrate nitrogen concentrations after 100 days are plotted in Fig. 3 against the total amount of carbon added with the standard 6 mg. of nitrogen. The results fall significantly on to a straight line such that 1 mg. of nitrate nitrogen is removed by about 30 mg. of carbon. At earlier periods there was no such regular relationship, showing that the systems were not even approaching equilibrium, as is indeed evident from the few curves plotted in Fig. 1 B. It follows from this slow approach to equilibrium that nitrogen in mixed organic manures applied in the field or in the mixtures formed by the added material and the decomposable organic matter already present as crop residues, will become available irregularly and that the course of the decomposition will be profoundly modified by comparatively small changes in environmental conditions. No very close agreement can therefore be expected between the results of experiments conducted under different conditions.

The nitrification experiments as a whole show (1) that tares liberates much of its nitrogen extremely rapidly, (2) that at first mustard locks up available nitrogen, (3) that both of these processes proceed more rapidly and completely with green manures alone than with mixtures in which part or all of the protein and cellulosic materials is derived from blood and straw respectively, and (4) that although these differences may depend in part on the actual compounds present, there is some evidence that the slowness and incompleteness of the decomposition of the mixtures depends on the less intimate association of the proteins and cellulosic bodies present in the mixtures.

To lock up equal amounts of nitrogen by mustard and by a mixture

of tares and straw requires a wider C : N ratio in the mixture than in mustard, and to secure equal liberation of available nitrogen from tares and from a mixture of mustard and blood requires a narrower C : N ratio for the mixture. The striking failure of tares in the Woburn field experiments is certainly not due to any resistance to nitrification; it may, on the other hand, be connected with its excessively rapid nitrification.
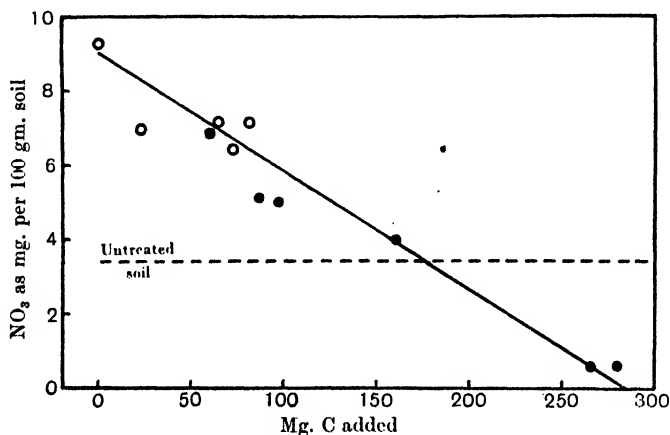
Fig. 3. Relationship between nitrate accumulation after 100 days and amount of carbon added with 6 mg. of nitrogen per 100 gm. of soil. Open circles: tares, blood and ammonium sulphate; dots: mustard, old and young.

POT-CULTURE EXPERIMENTS.

Pot cultures on wheat were conducted during 1929–30 at Woburn, using the same fallow-plot soil and the same forms and amounts of organic materials as in the laboratory experiments. The tares and mustard were finely ground air dried samples from the Stackyard field plots and were taken immediately before the folding by sheep in summer 1929. All additions supplied 6 mg. nitrogen per 100 gm. soil or 0·5 gm. nitrogen per pot, with amounts of carbon to give C : N ratios of 13 : 1 for tares, mustard + blood, straw + blood and of 26 : 1 for tares + straw, mustard, straw + blood.

There were three other series which received no additions: untreated fallow-plot soil was used to test the general effect due to the additions of organic matter, and untreated samples of soil from the unlimed portions of the continuous tares-wheat and mustard-wheat rotation plots in Stackyard field, Woburn were taken in October, 1929, during the cultivations for the wheat. Owing to the summer drought it had proved

impossible to grow the usual second crops of green manures, and the samples therefore contained the products from the decomposition of one crop of tares and mustard respectively during a dry period from the end of July to late October. In 1929 these soils differed from those in normal seasons in that at the time of sowing the wheat they contained no fresh tares or mustard material and no fresh sheep manure. It has been shown in the preceding paper that after folding off the tares and mustard on these plots at the end of July, 1929, the nitrate contents in the field increased rapidly on the tares plot and more slowly on the mustard plot. At the time of taking the soils for the pot experiments the three untreated soils therefore contained supplies of immediately available nitrogen with comparatively small reserves of plant residues.

The pots were made from earthenware drain pipes, 60 cm. deep and 15 cm. internal diameter, closed at the bottom by wire netting. They were filled by adding in turn, 1·5 kg. coarse gravel, 1 kg. fine gravel (passing 1 in. mesh), 8 kg. untreated soil and 9 kg. of soil containing the organic manures. All the soil had passed through a $\frac{1}{4}$-in. sieve and was added in 10 separate portions, each of which was lightly but uniformly pressed down. The water content was adjusted by weighings to 15·5 per cent. and maintained at this value by fortnightly waterings to constant weight with intervening waterings according to the estimated requirements. Distilled water was used throughout and the pots, though exposed in the open in the fine weather, were brought into the glass house during rain. There were 8 pots for each treatment (4 leached and 4 unleached). The 72 pots were randomised on trucks so as to eliminate place effects.

Twelve graded seeds of wheat—Little Joss W 3—were sown in each pot on November 11, 1929. From November 27 two-daily counts of the numbers of plants germinated were made until germination was complete. The results are given in Table III as percentage germinations and as the times required for three-quarters of the final number of plants to appear.

One half of each series of pots was leached with distilled water on six occasions between December 30, 1929 and April 16, 1930. The amount of water added for leaching was increased progressively as the plants gained strength, with a maximum of 3 litres of water; drainage proceeded for 2–4 days and the soil returned to normal moistness in about a week. On each occasion the leachate from each pot was measured, filtered and analysed for nitrate; tests for nitrite and ammonia showed that these were present only as minute traces (below 1 part of nitrogen

per million). One each of the untreated tares-plot and mustard-plot soils was damaged during the experiment but in the remaining 70 pots the growth proceeded quite normally throughout.

Thinning was postponed to a late date so as to reduce the risk of a loss of plants from temporary waterlogging during a hard winter. On February 28, 1930, the pots were watered and on the next day the plants were reduced to 6 per pot by pulling out the extra plants so as to remove as much root as possible. It was noticed that the plants in pots with mustard (both leached and unleached) had much longer and more abundant root systems than those with any of the other treatments. The plants from each treatment (separating leached and unleached) were cleaned, mixed, dried, weighed, and analysed for total nitrogen. By making the assumption that the plants removed were similar to those left in the pots it was possible to estimate the dry matter and nitrogen contents of the young wheat plants at the time of thinning (March 1).

Observations on the development of the plants taken periodically included frequent counts of the number of plants, and shoots per pot, and the height of each shoot to the base of the top unfolded leaf. In the statement of results the shoot numbers before thinning are reduced to a constant basis of 6 plants per pot. The shoot heights are recorded by giving the time at which half of the final value was reached. During the later stages of growth there were striking differences in the rates of ear emergence. Two-daily counts of the fully emerged ears were made, and the results are summarised by recording the times at which one-half of the final number of ears had emerged.

The wheat was harvested on August 18, 1930, and the produce of each pot was treated separately throughout in order that valid estimates of standard error could be made not only for yield of grain and straw (including chaff and husk) but for each of the quantities: number of ears, number of grain, nitrogen percentage and total nitrogen content of grain and of straw.

THE REMOVAL OF NITRATE BY LEACHING.

Table III gives the mean nitrate nitrogen content of each of the leachings. The standard error of the total nitrate was calculated for the fallow-plot soils only (28 pots) as one pot was lost from each of the tares-plot and mustard-plot soils.

The average total nitrate content of the leachates from the three sets with 13C : N was 85 mg. and that from the three with 26C : N was 60. The difference (25) may be regarded as significant, as the standard

error for these means of 12 pots is 5·8. Considering the individual results, it will be seen that both mustard and tares + straw significantly depressed the nitrate loss below that from the untreated soil. The other differences for the fallow-plot soil cannot be regarded as significant in view of the high standard error.

Table III. *Amount of nitrate nitrogen in mg. in pot in total leachate.*

| Leaching | | Soils from green-manure plots, No addition | | | Soil from fallow plot with added organic matter | | | | | |
| | | | | | +13C:1N | | | +26C:1N | | |
Date	Amount in litres	Tares plot	Mustard plot	No addition	Tares	Mustard +blood	Straw +blood	Mustard	Tares +straw	Straw +blood
31. xii. 29	1·0	14	6	2	3	2	3	2	3	2
17. i. 30	1·5	62	57	24	24	24	21	15	15	25
4. ii. 30	2·5	32	19	40	40	38	32	22	21	35
22. ii. 30	2·5	7	8	6	22	16	11	7	7	12
19. iii. 30	2·5	2	2	3	7	4	2	2	3	4
16. iv. 30	3·0	2	4	3	3	2	1	2	1	2
	Total	119	96	78	99	86	70	50	50	80

Standard error of the totals 8·2 (or 11·3 per cent. of the general mean).

Both the tares-plot and the mustard-plot soils gave very high nitrate contents, especially in the earlier leachings. The second leaching contained more than twice as much as that from any other soil and one-half of the total from these soils. As these were both leachings with small amounts of water, it is clear that at the time of sowing wheat there was much readily available nitrogen from both the tares and mustard folded off three months before.

The low nitrate contents of the last two leachings is ample evidence that by this time the combined action of plant and drainage had reduced the nitrate concentration to a very low level, and that any available nitrogen formed in March and April was immediately assimilated by the rapidly growing plants.

It should be pointed out that in a normal English autumn and winter extensive drainage commences much earlier than the leaching in these experiments and the losses of nitrate in the field are probably much more serious than in our pots.

THE DEVELOPMENT OF THE CROP.

Germination.

Within the first fortnight of the experiment differences between the tares and mustard treatments were apparent in the rates of germination which are recorded in a condensed form in Table IV. In all cases the

series with 13C : N ratios gave less complete and less rapid germination than those with 26C : N. Very soon afterwards, however, the plants with manures of the lower C : N ratio caught up with the other series and grew much more rapidly. Although it has not proved possible as yet to examine the effect on germination more closely, it has undoubted practical and scientific interest. It has been suggested to us by experienced observers that one of the reasons for the poorness of wheat after tares may be found in its poor germination owing to the dryness of the soil after tares. This explanation is obviously not applicable to our experiments, for special measures were taken to keep the surface of the soil uniformly moist during the period of germination. It is indeed possible that the poor germination observed by farmers is due to some such effect as that in our pot experiments. It is well known that the accumulation of carbon dioxide or ammonia may inhibit the germination of seeds and Fred (3) has shown that certain organic manures may have an adverse effect through causing a large growth of fungi. Although he did not find this effect with wheat, he did not experiment with tares which decompose with extreme rapidity and allow the accumulation of ammonia, carbon dioxide, and fungi.

Table IV. (a) *Final germination as percentage of seeds sown.*

	Uncropped soil			
	Green manures added	Green manure mixtures added	Straw + blood added	Field-plot soils No addition
13C : 1N, tares equivalents	86·5	84·4	84·4	81·9
26C : 1N, mustard equivalents	88·5	86·1	92·5	87·5
Difference 13C–26C	− 2·0	− 1·7	− 8·1	− 6·4

(b) *Time in days required for germination of 75 per cent. of final number of seedlings.*

13C : 1N, tares equivalents	18·6	17·8	17·3	16·8
26C : 1N, mustard equivalents	17·2	16·5	15·9	16·2
Difference 13C–26C	1·4	1·3	1·4	0·6

Tillering.

Shortly after germination the plants in the mustard series, whether leached or unleached, fell far behind the others. The plants were small single shoots with narrow leaves and a bluish grey colour. Growth and tillering proceeded actively in the unleached soils in the tares equivalent series and in the untreated soils and much more slowly in the leached

series. The whole course of development is illustrated in Fig. 4 for the leached and unleached tares and mustard treatments. With unleached tares tillering proceeded steadily in January and February and rapidly in March to a maximum of 6 shoots per plant, whereas with leached tares there was a much slower rise to a maximum of 3 per plant. With mustard tillering was very slow; until March there were no tillers in either series, and the maximum shoot numbers (2·7 per plant) were not reached until several weeks after the tares had reached its maximum.
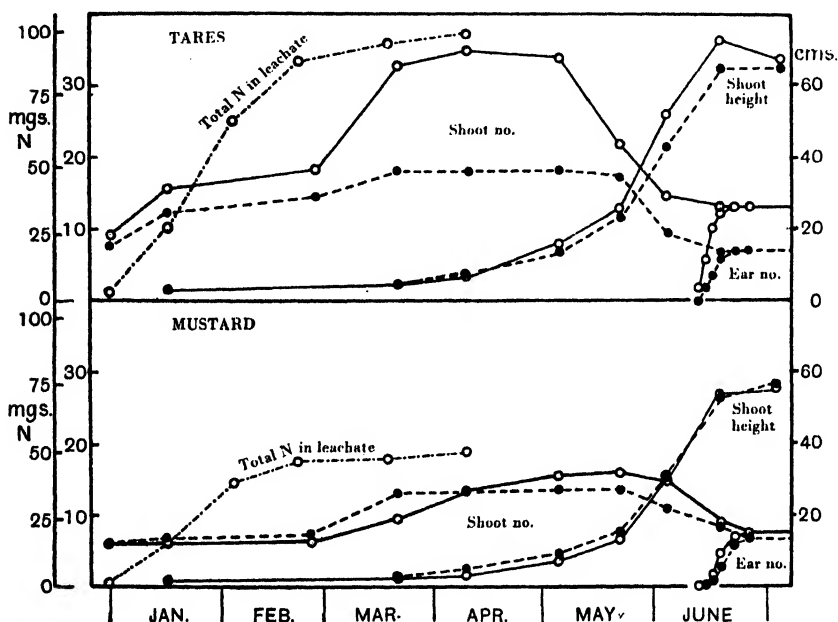
Fig. 4. Developmental data for wheat in pots manured with tares and mustard respectively. Full lines for unleached pots and broken lines for leached.

In the earlier stages the unleached series was actually below the leached series in tiller numbers. The shortage of available nitrogen in the mustard series is in harmony with the nitrate content of the leachings and is reflected in the low final ear numbers.

Mean values of the data for all of the treatments are given for several of the more important stages of development in Table V and for the final yields and nitrogen contents in Table VI. To facilitate comparisons between the different treatments at successive stages of growth, the general forwardness or backwardness of the plants is represented in Fig. 5 by plotting the deviations of the treatment mean from the general
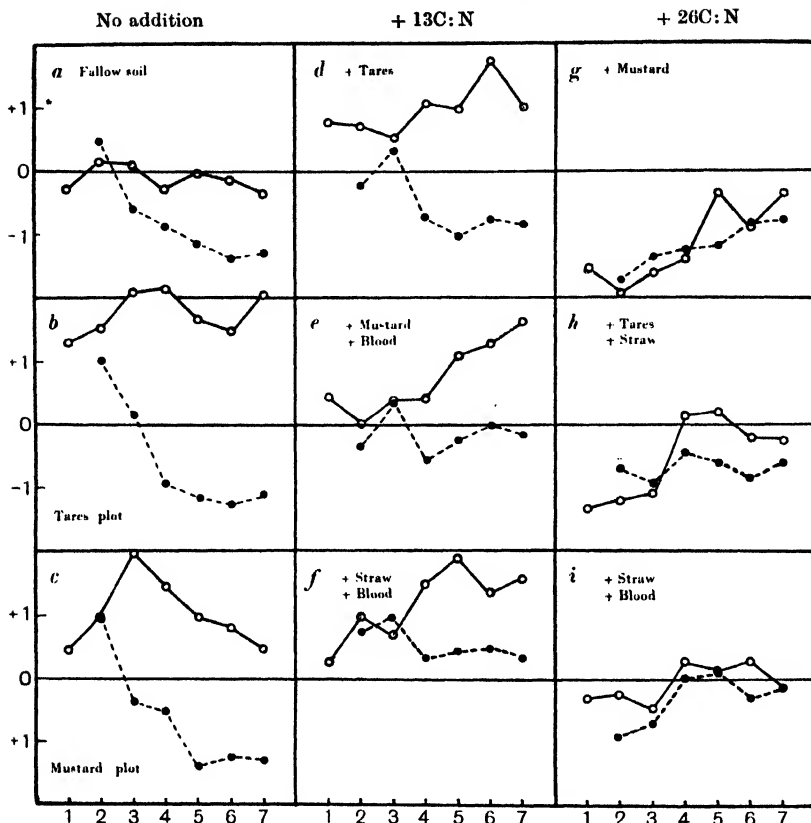
Fig. 5. Comparison of the rates of development and final yields of wheat in unleached pots (full lines) and leached pots (dotted lines) for (1) three series of soil without added organic matter, (2) three with added organic matter equivalent to tares, and (3) three with added organic matter equivalent to mustard. Deviations from general mean are given in terms of the standard deviation of the means for 18 treatments. Abscissae 2–6 represent successive stages of development as shown below.

	Date	Mean value M	Standard deviation S.D.	S.D. as % of M
1. Nitrogen in mg. in leachate and leached plants at thinning	1. iii. 30	97	28	29
2. Shoot numbers, early period, during leaching	1. i. 30–27. ii. 30	12·1	3·1	25
3. Nitrogen in mg. in young plants at thinning	1. iii. 30	24·6	12·1	44
4. Shoot numbers, middle period	22. iii. 30–6. v. 30	24·5	8·6	35
5. Shoot numbers, late period	23. v. 30–20. vi. 30	13·9	2·6	19
6. Grain and straw in gm.	18. viii. 30	25·8	7·3	28
7. Nitrogen in mg. in grain + straw	18. viii. 30	196	53	27

mean, as multiples of the standard deviation of the 18 treatment means. This makes it possible to compare dissimilar quantities such as tiller numbers and nitrogen contents on a uniform basis. The general means and standard deviations used in preparing Fig. 5 are given below the figure.

Some of the individual stages will be discussed first. In dry weights and nitrogen contents of the young plants at the time of thinning (Table V, rows 2 and 3; Fig. 5, abscissae 3), it will be seen that leaching had an appreciable effect only on the two untreated soils from the green manure plots. It has already been shown that these contained much larger amounts of immediately available nitrogen than the fallow-plot soils with added organic matter. Some of this immediately available nitrogen was thus removed before the young seedlings could absorb it. In all of the series in fallow-plot soil the leached plants had slightly more dry matter, but the same total nitrogen contents as the unleached ones. Although leaching had thus little or no effect on the absorption of nitrogen before March 1, there were striking differences between the effects of the added organic materials. Each of those with $13C : 1N$ gave a higher nitrogen content than the untreated soil and each of those with $26C : 1N$ gave a lower nitrogen content. Mustard treatment reduced the nitrogen content of the young plants to one-fifth of that of the unmanured plants when unleached and to one-half when leached. In all cases the amount of nitrogen in the young plants was less than half and in some as little as one-sixth of that removed in the leachate. This is one of the most important results of the experiment, for it amply demonstrates the inability of young wheat plants to utilise the large amounts of nitrogen often made available during the autumn and early winter. Under field conditions the losses are likely to be greater than in these experiments, for the cultivation for the wheat seed-bed favours active nitrification and leaching, whilst in these experiments the green manures were added at the time of sowing and leaching was not commenced until 7 weeks later.

By adding together the total nitrogen contents of the leached plants (including the ones removed at thinning) on March 1 and the total nitrogen removed in the leachates up to this date it is possible to obtain an estimate of the total available nitrate produced by this date. The results are given in Table V, row 4 and in Fig. 5, abscissae 1 (they differ but slightly from those given by adding rows 1 and 3 in Table V, for the amount of nitrate subsequently leached was small and of the same order as the nitrogen in the few plants removed at thinning). Mustard

Table V.

| | | Untreated-field soils | | | Uncropped-plot soil with added organic matter | | | | | | s.e. for (d) to (i) (figures in brackets give s.e. as % of general mean) |
| | | | | | 13C:1N | | | 26C:1N | | | |
		Tares plot (a)	Mustard plot (b)	Un-cropped plot (c)	Tares (d)	Mustard + blood (e)	Straw + blood (f)	Tares + straw (g)	Mustard (h)	Straw + blood (i)	(j)
1. Nitrate nitrogen in total leachate, mg.	L	118	96	78	99	86	70	50	50	80	8·2 (11·3)
2. Weight of plants left at thinning, gm.	U	1·04	0·91	0·46	1·10	0·85	0·85	0·37	0·33	0·48	—
	L	0·86	0·76	0·68	1·16	1·31	1·34	0·40	0·31	0·80	—
3. Nitrogen in plants left at thinning, mg.	U	50	45	26	30	29	33	12	5	19	—
	L	27	20	17	29	28	37	13	8	16	—
4. Nitrogen available by 1.iii.30 (nitrogen in total leached plants + leachate by 1.iii.30)	L	161	125	102	136	131	130	70	61	102	—
5. Shoot number, early period (1.i, 17.i, 27.ii)	U	16·8	15·0	12·6	14·3	12·1	15·1	8·4	6·1	11·3	—
	L	15·2	15·0	13·5	11·4	11·0	14·3	10·0	6·8	9·3	—
6. Shoot number, middle period (22.iii, 16.iv, 6.v)	U	42·7	36·9	21·9	33·9	27·8	37·2	25·4	12·7	26·7	2·15 (9·2)
	L	16·3	18·8	16·8	18·2	19·9	14·3	20·8	13·1	24·7	—
7. Shoot number, late period (23.v, 5.vi, 20.vi)	U	15·0	16·4	13·8	16·4	16·8	18·8	14·4	13·0	14·3	—
	L	10·9	10·2	10·9	11·2	13·3	15·0	12·2	10·7	14·2	—
8. Time for half shoot height (for days ahead of general mean)	U	-2·2	-2·3	-0·1	-2·5	-1·7	-1·7	+1·9	+2·5	+0·1	—
	L	+1·9	+2·9	+3·7	-1·2	-1·5	-3·6	+1·2	+3·1	-0·8	—
9. Time for half ear emergence (for days ahead of general mean)	U	-1·7	-1·0	-0·4	-1·8	-1·2	-1·5	+1·4	+1·5	-0·8	—
	L	+1·2	+1·8	+3·0	-0·5	-0·8	-1·6	+1·4	+3·0	-0·4	—
10. Number of ears	U	12·2	11·8	8·5	13·0	12·5	11·8	8·0	7·5	9·3	—
	L	6·0	6·0	6·0	6·5	8·8	11·0	6·8	6·5	7·3	—
11. Number of grain per ear	U	21·5	21·2	24·2	19·1	22·4	22·6	22·5	22·9	24·2	—
	L	23·3	23·0	23·6	24·7	21·1	21·0	23·2	24·8	26·2	—

All values as means per pot of 6 plants.

U = Unleached series; L = Leached series.

Table VI.

		Untreated-field soils			Uncropped-plot soil with added organic matter						
					13C:1N			26C:1N			s.e. for (d) to (i) figure in brackets give s.e. as % of general mean
		Tares plot (a)	Mustard plot (b)	Un-cropped plot (c)	Tares (d)	Mustard + blood (e)	Straw + blood (f)	Tares + straw (g)	Mustard (h)	Straw + blood (i)	(j)
1. Grain, gm.	U	10.6	11.2	8.8	10.0	11.4	11.3	8.0	7.7	9.8	0.35
	L	6.3	6.6	5.8	6.8	8.9	9.1	6.8	7.5	8.2	(4.0)
	U–L	4.3	4.6	3.0	3.2	2.5	2.2	1.2	0.2	1.6	—
2. Straw, gm.	U	25.9	20.2	16.9	28.5	23.9	24.4	16.2	13.5	18.0	1.10
	L	10.4	10.1	10.0	13.6	16.9	20.2	13.1	12.3	15.5	(6.1)
	U–L	15.5	10.1	6.9	14.9	7.0	4.2	3.1	1.2	2.5	—
3. Grain + straw, gm.	U	36.5	31.4	25.7	38.5	35.7	35.7	24.2	21.1	27.9	0.92
	L	16.7	16.7	15.8	20.4	25.8	29.3	19.7	19.9	23.7	(3.4)
	U–L	19.8	14.7	9.9	18.1	9.5	6.4	4.5	1.2	4.2	—
4. Nitrogen in grain, mg.	U	190	151	126	150	175	181	128	136	150	7.0
	L	105	104	97	111	144	148	118	121	136	(5.0)
	U–L	85	47	29	39	31	33	10	15	14	—
5. Nitrogen in straw, mg.	U	115	68	51	99	107	96	56	46	54	8.1
	L	34	24	31	43	44	63	47	35	51	(13.1)
	U–L	81	44	20	56	63	33	9	11	3	—
6. Nitrogen in grain + straw, mg.	U	305	219	177	249	282	277	184	182	204	18.0
	L	139	128	128	154	188	211	165	156	187	(9.0)
	U–L	166	91	49	95	94	66	19	26	17	—
7. Nitrogen per cent. of grain	U	1.82	1.36	1.35	1.51	1.53	1.61	1.60	1.65	1.53	0.015
	L	1.65	1.57	1.68	1.64	1.62	1.62	1.82	1.61	1.67	(0.90)
8. Nitrogen per cent. of straw	U	0.46	0.32	0.27	0.35	0.44	0.40	0.35	0.34	0.30	0.038
	L	0.31	0.24	0.31	0.32	0.26	0.31	0.34	0.29	0.33	(11.3)
9. Ratio of grain weight to grain + straw weight	U	0.29	0.36	0.34	0.26	0.32	0.32	0.33	0.36	0.35	—
	L	0.38	0.40	0.37	0.33	0.34	0.31	0.34	0.38	0.35	—
10. Ratio of nitrogen in grain to nitrogen in grain + straw	U	0.62	0.69	0.71	0.60	0.62	0.65	0.70	0.75	0.74	—
	L	0.76	0.81	0.76	0.72	0.77	0.70	0.72	0.78	0.73	—

All values as means per pot of 6 plants.

U = Unleached series; L = Leached series.

and (tares + straw) gave less available nitrogen, and each of the 13C : N series gave more available nitrogen than the corresponding untreated soil. The tares-plot soil gave considerably more early available nitrogen than the mustard-plot soil.

The forwardness and the healthy green colour of the plants in the green manure-plot soils and in the fallow-plot soil with tares equivalents were very striking at this stage for both leached and unleached soils. This is illustrated by the early shoot numbers (Table V, row 5, and Fig. 5, abscissae 2) which are high for these treatments and resemble the nitrogen contents of the young plants in showing no consistent leaching effect.

From the time of thinning the effects of leaching become much more striking in all of the series except those receiving organic manures with 26C : 1N. The rapid tillering illustrated in Fig. 4 for the unleached tares series also occurred in the unleached tares-plot and mustard-plot soils and in the other unleached 13C : N series, whereas the corresponding leached pots tillered much more slowly even though for the 13C : N series there was no difference in nitrogen content at the time of thinning. The effect of previous leaching is well shown in Figs. 5 D, 5 E, 5 F, by the increasing divergence of the curves for leached and unleached 13C : N series. The greatest difference between leached and unleached series was for the tares-plot soil. In the 26C : 1N series the differences between leached and unleached series were small and inconsistent. Both mustard series started off much below the others but gained steadily, until in final yields they were ahead of the leached tares and the three leached untreated series. The other series with 26C : 1N caught up with the general mean by the time of maximum tillering (abscissae 4). The actual numbers of shoots in Table V show that in the unleached tares-plot and mustard-plot soils and the unleached soil with added tares the late shoots were about equal to those of the early period, whereas in the leached soils the late shoots were less than those in the early period. In the unleached series enough nitrogen was available to carry to maturity most of the tillers originally formed, but in the leached series there was a nitrogen shortage in the later stages of growth. With mustard and with all of the mixtures (in both leached and unleached series) the late shoot numbers were greater than the early shoot numbers, indicating that the nitrogen shortage was less acute in the late than in the early stages of growth.

The use of shoot numbers as approximate measurements of the amount of nitrogen available to the plants is based on plant physio-

logical evidence that the nitrogen supply limits the production of meri-
stematic tissue when other environmental conditions are suitable for
growth. Up to the point of maximum shoot numbers nitrogen is
absorbed rapidly and stored temporarily in highly nitrogenous young
shoots, but when the supply of nitrogen to the roots falls below that
required to meet the requirements for the growth of the larger and older
shoots, nitrogen is translocated to them from the smaller shoots which
die off. The intimate connection between shoot numbers and the nitrogen
contents of the plants is illustrated for the present experiments in Fig. 6.
At the time of thinning the shoot numbers and the nitrogen contents
are very closely correlated, and again at harvest there is a fairly close
correlation between ear numbers and the total nitrogen in grain and
straw, though there is some evidence of an upper limit of 2 ears per
plant. The correlation for the mature plant is also expressed by the fact
that the nitrogen percentages of the grain and straw and the ratios of
grain to straw vary much less among themselves than do the actual
yields. We have no determinations of nitrogen contents between thinning
and harvest, but the correlation of maximum shoot numbers and final
nitrogen contents is in agreement with the view that the plant absorbs
substantially the whole of its nitrogen by the time of maximum tillering.

Fig. 6 also illustrates the important fact already mentioned that,
although no appreciable amount of nitrate was removed by leaching
after thinning, the effects of leaching were entirely masked at the time
of thinning and became prominent only several weeks later when the
plants had taken up much more nitrogen. The effects of leaching on the
subsequent availability of nitrogen appear to be more important than
the actual removal of nitrate, though it will be shown later that these
two effects are closely correlated under the conditions of these experi-
ments.

It was observed that where there had been an early supply of
available nitrogen (tares-plot soil, mustard-plot soil, and fallow-plot soil
with tares and in both leached and unleached series in each case) the
plants were ahead of the others in the length of the shoots and in time
of ear formation even though the leached pots gave much fewer shoots
and ears. When the data in rows 8 and 9 of Table V for the earliness or
lateness of shoot and ear formation were plotted on curves similar to
those in Fig. 5, they fell quite out of line with the other developmental
data. The relative importance of the early nitrogen and the total nitrogen
in determining the rate of development of the shoot is shown in
Table VII, which gives the correlation coefficients for the time of

emergence of half the ears and the nitrogen contents at the times of thinning and at harvest respectively. (The times for half-shoot height were closely correlated with those for half-ear emergence.)
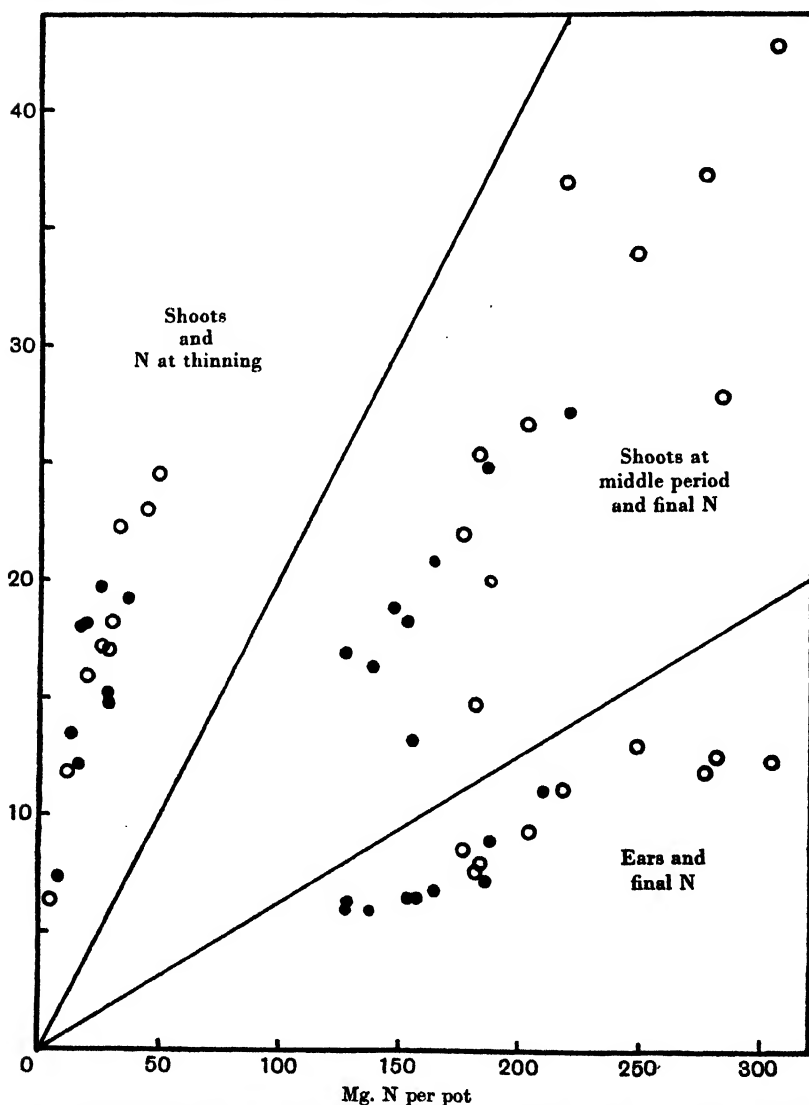
Fig. 6. Relationships between shoot numbers and nitrogen contents at different stages in the growth of wheat. Open circles for unleached pots; dots for leached pots.

Table VII. *Relationship of times of ear emergence to nitrogen contents of the plants.*

a = nitrogen content of plants per pot at thinning.
b = nitrogen content of plants per pot at harvest, grain + straw.
c = time of emergence of half of final number of ears (– equals ahead of general mean).

		Correlation coefficient			Percentage probability of similar effect by chance		
		All	Un-leached	Leached	All	Un-leached	Leached
r_{ab}	Early N and final N	+0·58	+0·71	+0·21	1	<5	High
r_{ac}	Ear emergence and early N	−0·78	−0·98 ⎫ *	−0·73	<1	<1	<5
r_{bc}	Ear emergence and final N	−0·72	−0·78 ⎭	−0·74	<1	<5	<5
$r_{ac \cdot b}$	Ear emergence and early N eliminating final N	−0·64	−0·96 ⎫ *	−0·88	<1	<1	<1
$r_{bc \cdot a}$	Ear emergence and final N eliminating early N	−0·53	−0·59 ⎭	−0·89	<5	High	<1

* The difference between these pairs of correlation coefficients is significant, *i.e.* ear emergence in the unleached series is connected more closely with the early nitrogen than with the final nitrogen.

The nitrogen contents at thinning and at harvest are correlated positively in the unleached series but not in the leached series. Early ear emergence is associated with high nitrogen contents both at thinning and at harvest, but the partial correlation coefficients show that in the unleached series time of ear emergence and early nitrogen are very highly correlated, and further that the time of ear emergence is more closely connected with the early than with the final nitrogen. In the unleached series supplies of nitrogen during active growth varied widely from treatment to treatment, but they were always adequate to allow the plant to develop at a rate determined by the initial supply. In the leached series the nitrogen subsequently became available at more widely different rates according to the treatments; the early and the final nitrogen contents were almost independent but they proved to be equally connected with the time of emergence of the ears. Low availability of nitrogen during the early stages was compensated for by greater supplies later from the soils which had lost least by leaching, whereas high initial availability gave large early plants whose subsequent development was retarded by nitrogen shortage later in growth.

STATISTICAL ANALYSIS OF FINAL YIELDS.

The experiment was designed primarily to test whether the essential differences between tares and mustard were to be ascribed to their elementary composition or to specific differences. These questions may be tested by averaging the results for the two C : N ratios and also by taking together the following pairs of treatments: (tares and tares + straw), (mustard + blood and mustard), (straw + blood (= T) and straw + blood (= M)). On the first hypothesis the effect of leaching should vary significantly with the C : N ratio, and there should be no significant interactions between each of the three main sources of organic matter and the effects of leaching, amount of carbon, and the interaction of leaching and amount of carbon and also no effect on the mean yield. Such tests are conveniently made by R. A. Fisher's Analysis of Variance which uses the degree of agreement between the replicates to estimate the probability that the observed differences may be ascribed to chance variations and experimental errors. In Table VIII the mean squares

Table VIII. *Analysis of variance of final yields.*

$e^{2z-2z'}$ where z' corresponds to $P = 0.01$.
Significant results italicised

	Degrees of freedom	Sums of squares (for mg. N in plant)	mg. N in			gm. dry matter in		
			Plant	Grain	Straw	Plant	Grain	Straw
1. Leaching	1	32,761	*3·4*	*4·6*	*5·2*	*24·6*	*11·9*	*7·9*
2. Amount of C	1	31,930	*3·3*	*3·3*	*4·3*	*30·1*	*8·2*	*13·4*
3. Leaching and amount of C	1	12,871	*1·3*	*1·0*	2·8	*8·5*	*2·0*	*3·8*
4. Form of C	2	9,057	0·7	*3·0*	0·2	*3·5*	*5·1*	*1·4*
5. Form of C and leaching	2	721	0·1	0·0	0·3	*2·3*	0·4	*1·5*
6. Form of C and amount of C	2	5,127	0·4	0·5	0·3	0·8	*1·2*	0·2
7. Form of C, amount of C and leaching	2	445	0·0	0·0	0·2	*2·2*	0·5	0·8
8. Error	36	46,916						
9. Total	47	139,828						
s.e. per pot		36·1						

due to treatments and to error are given for one of the variates tested (viz. nitrogen content of the grain and straw in mg. per pot), but instead of customary practice of recording the "z" values those for $e^{2z-2z'}$ are used. This quantity expresses the ratio of the observed mean square due to treatment to that required to give a 1 per cent. probability that the effect arose by chance. All values of $e^{2z-2z'}$ which exceed 1·0 may therefore be taken as highly significant, and those which exceed 0·7 ($P = 0·05$)

may also be regarded as significant. This method of expression has the advantage that it provides a ready means for comparing several variates from the same experiment in a simple table. The results of the analysis of the variance for the final yields of grain and straw and for grain + straw and for their nitrogen contents are given in Table VIII in this form. The analysis is restricted for simplicity to the 48 pots to which organic materials were added, and the standard errors for the means given in Table VI are subject to similar restrictions.

The results for total nitrogen in grain + straw are considered first. These show that both leaching and the C : N ratio have had highly significant effects and that the interaction between them is also highly significant. Inspection of the individual means in Table VI shows that leaching markedly depresses the nitrogen recovery from tares equivalents but not from mustard equivalents. This fully confirms the original hypothesis on which the work was based. Specific effects due to the various forms of carbon do not attain the level required for significance ($P = 0.01$), but there is an indication that the general level of nitrogen recovery depends on the form of carbon, and in the detailed results mixtures of straw and blood are generally better than tares or tares + straw. The analyses of the other yield data confirm those for nitrogen recovery. Owing to the better agreement between replicates the yields of dry matter show still more striking effects for leaching, amount of carbon, and their interaction. For every variate tested these effects are highly significant and always depend on the superiority of unleached tares equivalents over leached tares equivalents and over mustard equivalents, whether leached or unleached. There are in addition to this main effect a few significant specific effects which can be summarised as follows:

1. In the nitrogen content of grain, tares and tares + straw give lower values than other materials of the same C : N ratio whether leached or unleached.

2. In dry weight of grain, tares alone gives less than other materials of 13C : N whether leached or unleached.

3. In dry weight of straw, mustard alone and mustard + blood are less influenced by leaching than the other materials.

4. In dry weight of grain + straw, leaching has more effect on tares alone and less effect on mustard alone than on equivalent mixtures. The third and fourth results are in harmony with the original hypothesis with a modification brought out by the laboratory experiments, viz. that tares gives more and mustard less rapid and complete nitrifi-

cation than equivalent mixtures. The inefficiency of tares for grain production brought out in the first and second additional significant interactions is not expected from the original hypothesis, though it resembles one of the outstanding features of the Woburn field experiments. It also happens that untreated soil from the tares plot gives less grain than that from the mustard plot, but this difference is small and uncertain. Comparison with the untreated fallow-plot soil shows that in the unleached series the addition of tares increased the straw by two-thirds but the grain by only one-seventh. In each of the three sets, untreated soils, soils with addition of 13C : N, and soils with addition of 26C : N, the ratios of grain to grain + straw and of nitrogen to grain in nitrogen in grain + straw are lower for soils receiving tares in some form than for the other soils. In the unleached series with 13C : N the tares produced rather more ears than the other materials but 10 per cent. less grains per ear.

It is not possible from these experiments to explain the abnormality of tares when no leaching occurs. There are indications that it arises from an excessively rapid availability of nitrogen leading to too rapid development of the plants and an insufficiency of available nitrogen in the later stages of growth. It is, however, quite clear that the major difference between tares and mustard lies in the greater losses from tares through winter leaching and also that any additional effects are due to the abnormality of the tares rather than to an abnormality of the mustard as has sometimes been supposed in discussions on the Woburn results.

NITROGEN AVAILABILITY AND PLANT GROWTH.

The intimate relationship between the extent of loss of nitrate during winter leaching and the reduction in final crop yield or nitrogen recovery in these experiments is shown in Fig. 7 and in the following correlation coefficients and regression equations:

a = nitrate nitrogen in total leachate in mg. per pot.

b = nitrogen content of unleached crop less nitrogen content of leached crop in mg. per pot.

c = dry weight of grain + straw in unleached crop less dry weight of grain and straw in leached crop in gm. per pot.

$$r_{ba} = + 0.87; \ [b = 1.89 \ (a - 44.5): a = 0.40 \ b + 63];$$
$$r_{ca} = + 0.92; \ [c = 0.267 \ (a - 44.2): a = 3.15 \ c + 50].$$

The correlation coefficients are highly significant and the regression lines bring out the important result that the adverse effect of leaching

on the final plant is not proportional to the amount of nitrate removed. About 50 mg. of nitrogen per pot may be removed without reducing the crop, but as the nitrogen loss increases above this amount the nitrogen content of the plant falls off about twice as rapidly. This is clear proof that early nitrification and leaching lead to an indirect loss of available nitrogen which is much greater than the actual amount of
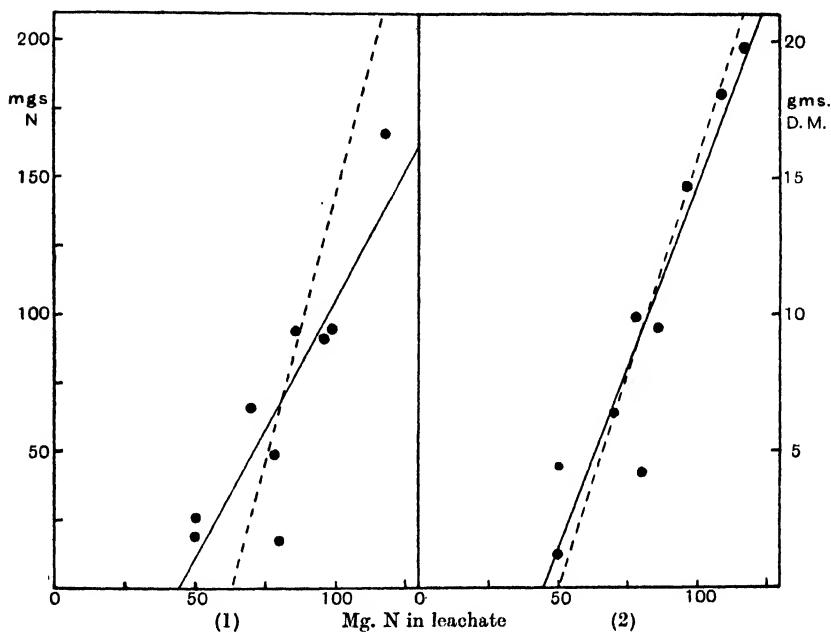
Fig. 7. Relationship of the nitrate in leachate and the reduction by leaching of (1) nitrogen content of grain + straw, and (2) dry weight of grain + straw.

extra nitrate lost. For spring applications of sodium nitrate or ammonium sulphate the recovery of nitrogen by wheat rarely exceeds one-third and for autumn applications is generally still lower. Nitrate produced from green manure residues thus behaves quite differently from nitrate from artificial fertilisers.

Although the present series of experiments was devised to measure the extent and effect of the loss rather than to throw light on its mechanism, it is appropriate to refer here to one or two of the hypotheses now being examined in extending this work. The better root systems of the young plants in the soils with low initial nitrate contents may enable them to utilise later supplies more effectively. The well-established

but unexplained loss of total nitrogen from cereal soils may be greater in soils producing appreciable amounts of nitrate in autumn and early winter. We are of opinion, however, that the principal factor is the influence of the added organic matter and the leaching on the competition between the plant and the soil micro-organisms at different stages during the growth of the plant. The three untreated soils used in our experiments contained relatively large amounts of available nitrogen and relatively little available carbon compounds, for there had been sufficient opportunity during three months fallow in a dry spell to allow oxidation without leaching. The yields in the unleached soils showed that the reserves of available nitrogen increased in the order fallow plot, mustard plot, tares plot, as would be expected. Leaching reduced this immediately available nitrogen, and in the richer soils the leached plants had less nitrogen than the unleached, even during the period of leaching. The low nitrate content of the soil would retard further oxidation of the carbon compounds of the soil sufficiently to allow it to continue in spring and early summer and so cause serious competition for nitrogen between the plant and the micro-organism during the critical period of active growth of the wheat.

When tares were added to unleached soil there would be a considerable liberation of ammonia and nitrate, but much of the nitrogen would be locked up again in the decomposition of carbon from the tares and from the soil. This decomposition would proceed rapidly at first in the presence of much readily available nitrogen, but later, when most of the carbon compounds were oxidised and the plant was able to assimilate large amounts of nitrate, competition with the micro-organism would be reduced. Leaching the soil treated with tares reduced the nitrate concentration, but the liberation of ammonia and nitrate was sufficiently rapid to meet the small requirements of the young plant, for there was no appreciable effect of leaching on the nitrogen content of the young plants at the time of thinning. The decomposition of carbon compounds would, however, be retarded by the removal of nitrate sufficiently to increase the assimilating power of the micro-organisms during the critical period of rapid nitrogen uptake by the wheat. Although some of the micro-organic proteins would ultimately yield nitrogen available for the plants, much of it would be too late to be fully utilised for growth and the total reduction by leaching would be greater than the amount of nitrate actually found in the drainage water.

Mustard provided insufficient nitrogen for the decomposition of the carbon compounds added, and the immediate supply of ammonia and

nitrate was greatly reduced for a long period. Ultimately when the excess carbon had been oxidised more nitrogen would be provided for the plant. It appears that in the present experiments the extra late nitrogen just counterbalanced the effect of the initial shortage of nitrogen. For low initial nitrate contents leaching could have little effect on nitrate assimilation by the plant or by the micro-organisms.

The plant development curves show very clearly the progressive decline of plants in the leached tares series and the steady advance of those in both the mustard series relative to the general means. Although the leached tares treatment did not prove significantly poorer than the leached mustard series, there are indications that this result might have been obtained if the experiment had been commenced earlier to provide greater opportunity for nitrification and leaching before the wheat was sown. In the present experiment nitrification and growth proceeded for 6 weeks before the leaching was commenced. Although the soil from the tares-plot soil was richer than the fallow-plot soil treated with tares under unleached conditions, it suffered so much more from leaching that it gave a lower yield and nitrogen recovery in the leached series. Although there was less initial available nitrogen in the fallow-plot soil with tares, the fresh organic matter was able to retain more nitrogen for the critical early summer period. Additional support for this explanation of the discrepancy between the pot experiments and the usual field results is provided by the exceptional results of 1928-9 in the Woburn field experiments. In Lansome field the wheat followed immediately after the ploughing in of the green manures, and the winter was cold and dry. The yields were exceptional in that they approached those of wheat in commercial cropping and tares gave better wheat than mustard. The conditions and the results resemble those of our unleached experiments. But in Stackyard field in the same season there was a 3 months' interval with much rain between folding of the green manures and drilling the wheat. The yields after both tares and mustard were extremely low and the tares plot gave the lower yield. The conditions resemble those in our leached-tares and mustard-plot soils which gave the lowest yields of the whole experiment. Our series with leaching after tares and mustard additions fell between the conditions of the two 1928-9 field experiments and tares proved about equal to mustard but better than no addition.

Loss of nitrate by leaching from light land is not confined to the winter months and in a two-course green manure-wheat rotation may be considerable during the 9 months of the green manure shift in which

34-2

the soil is either bare or carrying a young crop as well as in the 5 winter months in which the ground is occupied by young wheat. Although the tares plot receives nitrogen from the air and accumulates some of it as humus, and although the mustard plot carries over nitrogen from the autumn to the end of the following summer or later, there is still ample opportunity for the loss of the extra available nitrogen before the next wheat crop is able to use it. Further, such losses should become progressively worse, for they are accompanied by rapid oxidation of carbon compounds and so decrease the ability of the soil to resist subsequent leaching. We suggest therefore that the lack of adjustment between the time of producing and the time of utilising the soluble nitrogen in such rotations is the principal factor in the exhaustion of the Woburn green manure plots.

Better ways of utilising the characteristic effects of tares and mustard on light soils in mild wet climates may be suggested. Where tares can be grown successfully as an autumn-sown crop to stand the winter it will provide an abundant supply of readily available nitrogen for crops sown in spring shortly after ploughing in either the whole crop or its residues after folding. It is, however, essential that the tares crop should be sufficiently far advanced by early winter to prevent much loss of nitrate in drainage. A poor tares crop wastes available nitrogen; only a good one can add available nitrogen. The nitrogen of tares must be utilised almost as if it were given as an artificial fertiliser; tares crops in spring or early summer should be followed by rapidly growing crops such as roots, cabbage, kale or other fodder crops[1]. Late summer crops may be wasted if followed by late sown and slow-growing winter cereals on light freely draining soil, but appreciable amounts of the nitrogen may be utilised and conserved by winter rye or barley which may be sown earlier and grow more rapidly. Experiments are now in progress to ascertain whether the incorporation of straw before sowing wheat after tares can also be used to carry over available nitrogen from autumn to summer.

Mustard should be regarded as a means of locking up nitrogen and liberating it again some months later. It therefore forms a useful catch crop for summer and autumn. Again it is necessary to have good crops; starved ones provide too great an opportunity for loss of nitrate by

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[1] Compare Arthur Young, *The Farmer's Calendar*, 10th edition, 1815, 458: "A good crop of winter tares leaves the ground in such loose, putrid, friable order, that it is better husbandry to sow turnips or plant cabbage on it, than to leave it to receive tillage for wheat."

drainage, especially when they are grown frequently on the same land.

These suggestions are applicable only to light soils in regions in which the climatic conditions allow abundant drainage at all times. In heavier soils or with lower rainfall or lower winter temperatures drainage is less and the differences between tares and mustard are less marked. With heavier soils and drier climates the physical effects of the organic matter, both fresh and humified, become more important. Under such conditions the actual additions to the soil, carbon for all green manures and nitrogen as well for leguminous ones, may become the primary factors and the composition of the green manures only secondary.

DISCUSSION.

Although we are not as yet in a position to extend this interpretation of the action of green manures by actual measurements of the amounts of readily oxidisable carbon and nitrogen under conditions sufficiently similar to those in the field, we would suggest that the conception of a labile equilibrium between them and consideration of the effects of displacements in this equilibrium on the time at which soluble nitrogen is produced will facilitate the interpretation of a number of light land problems relating to residual effects of fertilisers, organic manures and other crops on succeeding crops. The "humus content" of the soil is of little importance in this connection, for the organic materials which are readily extracted or oxidised *in vitro* are almost inert in the biochemical changes involved in the production and assimilation of available nitrogen. Until it becomes possible to eliminate the nitrifying organisms by differential sterilisation, it appears necessary to maintain such ratios of available carbon to available nitrogen as will reduce nitrate production to a minimum except when there is an actively growing crop to take it up. Further, that ill-defined "condition" or "good heart" which is so highly prized by the farmer may depend on having sufficient reserves of available carbon and nitrogen in the soil to "buffer" it against too violent fluctuations in their ratio on the addition of more organic matter or fertilisers or on the removal of ammonia and nitrates by the plant or by leaching. The "slow and steady" action of nitrogenous fertilisers desired by farmers probably depends more on the previous history of the soil than on the specific properties of the fertiliser.

The Norfolk four-course rotation (roots, barley, seeds, wheat) provides an excellent illustration of the adjustment of the additions of carbon and nitrogen compounds to provide nitrogen to the crops at the

best times. In only one autumn in four is the soil cultivated for a slowly growing winter crop (wheat), and it is recognised that after this the soil needs heavy dressings of dung to restore its fertility. In the other autumns there is either an actively growing crop (roots and seeds ley) or the residues of a stubble rich in carbon. Fear of soil exhaustion was the principal ground for the former restrictions on cropping which prevented the taking of consecutive corn crops, *i.e.* of leaving the soil either bare or with a very small crop through two winters. This method of reducing the buffering with readily decomposable carbon and nitrogen compounds is, however, deliberately adopted when it is desired to have an early flush of nitrate with little late nitrate for barley intended for malting. Fallowing reduces the available carbon compounds and gives a temporary accumulation of available nitrogen and therefore a better crop immediately following the fallow, but the gain is at the expense of the general level of soil fertility. It may be suggested, too, that the merit of "sheeping" lies partly in maintaining a growing crop well into the winter and partly in compacting the soil and thus reducing the rate of percolation and providing more opportunity for the assimilation of soluble nitrogen compounds by the soil organisms.

The art of maintaining the fertility of light land in wet temperate regions involves in addition to supplying adequate amounts of lime, phosphoric acid, and potash, the maintenance of sufficient reserves of decomposable organic matter, with such an adjustment of the balance between available nitrogen and carbon as will lock up nitrogen in insoluble forms except when it is required by an actively growing crop. This balance may be secured by adding organic matter of relatively high $C : N$ ratio in the autumn (*e.g.* cereal stubbles, straw, sugar beet tops), by having actively growing crops in the autumn and early winter (*e.g.* temporary leys, trefoil, roots, kale, rye, or even weeds), and by adding both nitrogen and carbon by leguminous plants in leys. Experiments are being undertaken at Woburn to test these possibilities.

Summary.

1. It is suggested that the striking failure of winter wheat grown in rotation with two summer crops of tares or mustard on the sandy soil of the Woburn Experimental Station is due to the production of nitrate and ammonia from the green manures at times when the wheat is unable to use them efficiently and to the consequent loss of nitrate in the drainage. Owing to its low $C : N$ ratio the nitrogen in tares nitrifies very rapidly and the loss by leaching is very great. Mustard, on the other

hand, reduces the winter loss, but the nitrogen present in the mustard and that absorbed in the decomposition of the excess carbon compounds are liberated too slowly to be utilised efficiently by the wheat and much of the nitrate subsequently produced is also lost by leaching.

2. Nitrification experiments in the laboratory and pot experiments on wheat showed that nitrogen was made available more rapidly and more completely from materials with 13C : 1N (tares, mustard + blood, straw + blood) than from those with 26C : 1N (tares + straw, mustard, straw + blood). The yields in unleached pots were much higher with materials with 13C : 1N or in untreated soil, but in pots leached systematically during the winter the two types of organic matter were equally effective in increasing the yield. The reduction of crop by leaching was closely correlated with, but not proportional to, the extent of early nitrate formation as measured by the amount of nitrate leached from the pots. It is suggested that early nitrate formation reduces the yield not only by increasing the removal of nitrate by leaching but also by increasing the amount converted by the soil micro-organisms into forms which become available again only very slowly.

3. Tares material formed nitrates and mustard material removed it more rapidly and completely than equivalent mixtures. Under all conditions tested tares material proved rather less effective for grain production than the other materials. The less intimate association of the proteins and cellulosic substances in the mixtures appears to be sufficient explanation of these differences. There was no evidence of specific toxics or stimulants in mustard or tares.

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THE VALUE OF TAPIOCA FLOUR AND SAGO PITH MEAL IN THE NUTRITION OF SWINE.

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INTRODUCTION.

(1) *Tapioca flour.*

TAPIOCA flour, sometimes referred to as manioc meal, is now a comparatively well-known feeding stuff. Its value as a food for pigs has been tested in several recent farm feeding trials in this country and on the Continent[1, 2, 3], and, as a consequence, its merit, when used in partial replacement of barley or maize, is now generally recognised. Sago pith meal, on the other hand, has not hitherto been used in pig-feeding in this country, and no information respecting its composition and feeding value is available.

Tapioca flour comes on to the market as a nearly-white, finely-divided powder. Fullerton[1] gives the following information about its origin and production: Tapioca, cassava or manioc is the tuberous product of two species of plant belonging to the natural order Euphorbiaceae, viz. *Manihot utilissima* (bitter cassava) and *Manihot palmata* (sweet cassava). Both plants are native to tropical countries, Brazil, India and the East Indies being three of the countries in which they are found. The bitter cassava contains a poisonous juice which must be expressed before the root can be of any use. The residue of the root is then finely ground and sifted to give various grades of meal. Of these there would appear to be four: (1) Ground roots. (2) "C" tapioca, which is, apparently, partially screened meal. (3) "B" tapioca, made from the roots with the rind or bark removed. (4) "Ground Ampas," which is the ground up bark or rind. Of these grades, "B" tapioca is undoubtedly the best, and it was on this product that the trials at the Harper Adams Agricultural College were carried out.

Greenstreet[4], discussing the cultivation of the tapioca tuber in Malaya, noted the existence of the sweet and bitter varieties of tapioca,

the latter containing significant amounts of cyanogenetic glucosides. While these were present in the bitter variety, both in the flesh and the cortex, the sweet variety contained the poisonous substance only in the cortex. There would appear to be no vegetative characteristics to distinguish between the two varieties, and, in fact, the nature of the tuber seemed to depend on the locality in which it was grown.

That no harmful results are to be anticipated from the use of tapioca flour, in respect of the possible presence of traces of cyanogenetic glucosides, is evident from the fact that this feeding stuff is being used extensively for the feeding of pigs in this country, Denmark, Sweden and elsewhere, and that no cases of ill-health or poisoning in animals receiving the food have come to light. Indeed, tests carried out on the product used in the present trials revealed the presence of minute traces of cyanogenetic glucoside. Into each of six small flasks was weighed 5 gm. of tapioca flour. The material was then well mixed with 20 c.c. of distilled water and the flasks were corked. Suspended from each cork, inside the flask but not touching the glass, was a strip of moist sodium picrate paper. The flasks were then placed in an incubator at 37° C. After about 3 hours, the lemon-yellow tint of the sodium picrate paper had changed in every case to a faint orange-brown, an indication of the formation of a trace of prussic acid during incubation. (Note: In a blank test with distilled water alone, no colour change occurred.) That the amount of cyanogenetic glucoside in the tapioca flour was quite negligible, however, from the standpoint of possible toxicity, is shown by the observation that the faint orange-brown colour was not intensified after a further 20 hours' incubation. In the test employed, the paper should be turned deep brick red by prussic acid in 0·001 per cent. concentration. Indeed, in seeking to confirm the finding of the sensitive sodium picrate test by means of the less sensitive Prussian blue reaction, a negative result was obtained.

(2) *Sago pith meal.*

Sago pith meal is the broken and dried pith of the sago palm (*Metroxylon sagu*), which grows in many parts of Malaya, notably in Borneo, whence most sago products are imported. The largest sago districts in Sarawak are Mekah, Oya, Bintulu and Matu. Sago pith meal, therefore, is essentially an Empire product[1].

[1] For these details respecting the origin of sago pith meal, the writers are indebted to Messrs J. H. Z. Stallman (London), who kindly supplied the product for investigation, and also to the Malayan Information Agency, Malaya House (London).

The sago palm grows to a height of 30 to 35 ft. and attains a diameter of 2 ft. or more. During the growth of the palm, the pith gradually becomes filled with reserves of starch. The latter would be used up if the palm were permitted to flower. Just before the time of flowering, therefore, the palm is cut down and sawn into suitable lengths for handling. The outer woody layer, $\frac{1}{2}$ to $\frac{3}{4}$ in. in thickness, is then removed and the pith is broken down by mechanically driven rasps. The broken pith is sifted to remove the fibrous material of the vascular bundles. It is then dried by exposure to the rays of the sun or, alternatively, placed in a chamber in which the drying process is accomplished by means of warm air. The product is then ready for shipment.

Writing in November, 1929, Messrs Stallman stated that an initial output of about a thousand tons of sago pith meal per month should be available from Malaya, with a prospect of increasing to double this quantity. On first view, the product suggests powdered cork in appearance; actually, however, it is very different. It is a light-brown product composed mainly of powdery material which tends to "ball" together into tiny lumps, but which easily rubs out again into powder. It includes a good proportion of dusty material and a small fraction of thin fibrous pieces up to $\frac{1}{2}$ in. in length. The latter, however, are probably not an essential constituent of sago pith meal, but appear to be present owing to incomplete removal of fibrous material during sifting.

The experiments to be described in the present investigation were carried out with a view to securing data respecting the digestibility of tapioca flour and sago pith meal when fed to pigs. Large-scale feeding trials with pigs were also carried out, in order to test the validity of the conclusions from the digestion trials under conditions of farm-feeding. In the case of sago pith meal, such farm trials were being conducted for the first time; with tapioca flour, however, it was possible to compare the results with those obtained in similar farm trials at other centres (1, 2, 3).

I. DIGESTION TRIALS.

Two pure-bred, Large White hogs, weighing 195 and 188 lb. respectively at the commencement of the experiment, were used for the purpose of the digestion trials. In the first period of feeding, the digestibility of a ration composed of fish meal, middlings, maize meal and sago pith meal was determined. This was followed by a digestion trial of the basal food, composed of fish meal, middlings and maize meal, whilst in the final period of feeding, the sago pith meal of the first period was replaced

by an equal weight of tapioca flour. The details of the experimental rations are recorded in Table I.

Table I. *Details of digestion rations.*

	Period 1		Period 2		Period 3	
	Amount per day gm.	Dry matter per day gm.	Amount per day gm.	Dry matter per day gm.	Amount per day gm.	Dry matter per day gm.
Fish meal	150	132·50	228	201·64	150	134·37
Middlings	500	432·65	760	656·64	500	440·60
Maize meal	700	619·57	1064	931·85	700	618·10
Sago pith meal	700	608·44	—	—	—	—
Tapioca flour	—	—	—	—	700	618·03
Total	2050	1793·16	2052	1790·13	2050	1811·10

The rations were soaked overnight in water before being fed to the pigs, the amounts of water required being 5000 c.c. in period 1, 4000 c.c. in period 2 and 4500 c.c. in period 3. No difficulty of any kind was encountered in securing ready and complete consumption of the rations in all three periods, and the faeces of the experimental animals were of normal consistency throughout. In periods 2 and 3, the ration was given in three equal meals during the day. In period 1, however, on account of the somewhat greater bulk of the ration containing sago pith meal, it was found advisable to increase the number of meals during the day to four. It will be noted that the rations were so designed that the weight of food consumed daily by the pigs was very similar in the different periods, and that the basal ration in period 2 had the same percentage composition as the mixture of fish meal, middlings and maize meal contained in the rations of periods 1 and 3.

In Table II are recorded the results of an investigation into the swelling power of the different foods which were contained in the rations, the results for a sample of dried sugar beet pulp being also included for comparison. The experimental technique was substantially the same as that described by Procter and Wright(5). The tests on the maize meal, middlings and tapioca flour were carried out on the materials as purchased; in the case of the sago pith meal and sugar beet pulp, however, it was necessary to reduce the foods to a finer state of division before the tests could be carried out satisfactorily. In every case, 10 gm. of the feeding stuff was weighed into a measuring cylinder and, after tapping the cylinder, the "dry" volume was read off. Distilled water at 38° C. was added to bring the volume to 180 c.c., the water and feeding stuff being thoroughly admixed by stirring. The cylinders were then placed in an incubator at 38° C. and the volumes occupied by the

wet food were noted at stated intervals. The results of the observations are given in Table II.

Table II. *Swelling power of feeding stuffs used in digestion trials, with results for sugar beet pulp for comparison.*

| | "Dry" volume of 10 gm. of food c.c. | Volume of wet food after | | |
		2 hours c.c.	6 hours c.c.	24 hours c.c.
Maize meal	16	25	25	25
Middlings	26	46	46	45
Tapioca flour	21	30	30	—†
Sago pith meal*	28	38	38	40
Dried sugar beet pulp*	24	82	87	85

* After grinding. "Dry" volume of 10 gm. before grinding: sago pith meal, 36 c.c.; sugar beet pulp, 50 c.c.
† The tapioca flour had risen to the surface of the water after 24 hours.

The data in Table II show that tapioca flour is not a bulky food either in the dry or the soaked condition. Sago pith meal, before grinding, is fairly bulky in the dry condition, 10 gm. of the food occupying more than twice the volume (36 c.c.) than is taken up by an equal weight of maize meal (16 c.c.). The extent to which it swells when in contact with water, however, is not very pronounced, being somewhat less marked than with middlings. The extreme bulkiness of sugar beet pulp is strikingly brought out by the figures in the table. It is also interesting to note that in all cases, swelling was almost complete after 2 hours at 38° C., an observation which suggests that very prolonged soaking of meals before feeding to pigs is really not necessary.

The experimental periods of the digestion trials, during which the excreta were collected quantitatively for analysis, were of 10 days' duration. The harness and metabolism crates which were used to make possible the separate collection of urine and faeces have been described in a previous communication[6]. The various foods were sampled for analysis at the commencement of the feeding trials. Prior to each digestion period, the rations for the whole period were weighed into paper bags and at the same time, samples were taken for determination of moisture content. The composition of the different feeding stuffs is shown, on the basis of dry matter, in Table III. From the data in Table I, it is possible to calculate the moisture content of the feeding stuffs at the times of weighing out the rations.

Table III. *Composition of feeding stuffs (on basis of dry matter).*

	White fish meal %	Middlings %	Maize meal %	Tapioca flour %	Sago pith meal %
Crude protein	72·05	20·67	12·04	2·06	1·94
Ether extract	4·06	5·01	4·51	0·54	0·44
N-free extractives	0·73	62·18	78·33	92·12	88·71
Crude fibre	—	7·41	3·63	2·83	4·97
Ash	23·16	4·73	1·49	2·45	3·94
Silica	—	—	—	—	2·09
Silica-free ash	—	—	—	2·45	1·85
Lime (CaO)	—	—	—	0·25	0·38
Phosphoric acid (P_2O_5)	—	—	—	0·29	0·13
Potash (K_2O)	—	—	—	1·18	0·62
Soda (Na_2O)	—	—	—	0·02	0·28
Magnesia (MgO)	—	—	—	0·15	0·15
Chloride (Cl_2)	—	—	—	0·02	0·65

Comments on Table III.

The data for the composition of the sample of tapioca flour used in the present trials compare very satisfactorily with those given by Nils Hansson(3) for the same feeding stuff as employed in recent farm-feeding trials, the Swedish figures, on the basis of dry matter, being 2·06 per cent. of crude protein, 0·70 per cent. of ether extract, 92·31 per cent. of N-free extractives, 2·45 per cent. of crude fibre and 2·48 per cent. of ash. The present sample is also very similar in composition to that used in the Harper Adams College trials(1), the data for the latter, on the basis of dry matter, being 3·5 per cent. of crude protein, 0·7 per cent. of ether extract, 91·7 per cent. of N-free extractives, 1·9 per cent. of fibre and 2·2 per cent. of ash. It will be noted that the Cambridge sample was somewhat poorer in protein and slightly richer in fibre, but that the percentages of N-free extractives in both samples were almost equal.

Tapioca flour is obviously a particularly unbalanced food, containing extremely low percentages of protein, oil and ash and an unusually high percentage of carbohydrate, the latter being composed mainly of starch. It is designed solely, in respect of pig-feeding, to replace cereals and potatoes, although its protein content is lower even than that of these feeding stuffs. The mineral fraction of tapioca flour is richer in lime and poorer in phosphoric acid than the ash of wheat, barley or maize, the last-named, for example, containing 0·02 and 0·7 per cent. of these constituents. Tapioca flour, therefore, is better balanced than the cereals just mentioned in respect of its lime and phosphorus content, a comment which also holds true if tapioca flour is compared with potatoes. As with the cereals, tapioca flour is markedly deficient in chlorine and soda, but contains a relatively high percentage of potash. These findings are

of little practical significance, however, if the rations of pigs contain a mineral-rich food, such as white fish meal, or a suitable mineral supplement.

In many respects, sago pith meal resembles tapioca flour in composition. It is nearly as rich in carbohydrate, and of its 89 per cent. of this constituent, about 78 per cent. is in the form of starch (dry matter basis). Its content of protein and oil is unusually low, the percentages being of the same order as for tapioca flour. It appears to be of a more fibrous nature than tapioca flour, this being a consequence of the thin fibrous fragments which were found admixed with the powdery material in the sample of the food. It also contains a higher percentage of ash, although, on account of the fact that rather more than half of the total ash is composed of silica, its content of SiO_2-free ash is actually lower than that of tapioca flour. It will be noted from Table III that the ash of sago pith meal is distinguished from that of tapioca flour, and also of cereals, by its relative richness in chlorine. It is also well supplied with potash, but not quite so abundantly as tapioca flour. In contrast with the latter, it is richer in lime and soda, but poorer in phosphoric acid.

Table IV. *Digestibility of basal food (digestion period 2).*

	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.
			Pig VIII.				
Fish meal	201·64	154·94	145·28	8·19	1·47	—	46·70
Middlings	656·64	625·58	135·73	32·90	408·29	48·66	31·06
Maize meal	931·85	917·97	112·20	42·03	729·91	33·83	13·88
Total consumed	1790·13	1698·49	393·21	83·12	1139·67	82·49	91·64
Total voided	338·22	281·57	48·00	18·35	153·91	61·31	56·65
Total digested	1451·91	1416·92	345·21	64·77	985·76	21·18	34·99
Digestion coefficients of basal food, %	81·11	83·42	87·79	77·92	86·49	25·67	38·18
			Pig IX.				
Total consumed (as above)	1790·13	1698·49	393·21	83·12	1139·67	82·49	91·64
Total voided	319·45	267·17	45·25	18·62	140·55	62·75	52·28
Total digested	1470·68	1431·32	347·96	64·50	999·12	19·74	39·36
Digestion coefficients of basal food, %	82·16	84·27	88·49	77·59	87·67	23·93	42·95

Comments on Table IV.

It will be noted from Table IV that the agreement between the digestion coefficients of the basal food in the two separate animal experiments was quite satisfactory. It is further satisfactory to record the agreement of the mean digestion coefficients in the present trial

with the results obtained in an earlier trial with another pair of Large White hogs on a ration of the same composition, the mean digestion coefficients in the earlier trial being: total organic matter, 84·1 per cent.; crude protein, 86·8 per cent.; N-free extractives, 88·2 per cent.; crude fibre, 24·9 per cent.(7).

Table V. *Digestibility of tapioca flour (digestion period 3).*

	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extrac- tives gm.	Crude fibre gm.	Ash gm.
			Pig VIII.				
Fish meal	134·37	103·25	96·81	5·46	0·98	—	31·12
Middlings	440·60	419·76	91·07	22·07	273·97	32·65	20·84
Maize meal	618·10	608·89	74·42	27·88	484·15	22·44	9·21
Tapioca flour	618·03	602·89	12·73	3·34	569·33	17·49	15·14
Total consumed	1811·10	1734·79	275·03	58·75	1328·43	72·58	76·31
Total voided	241·00	201·56	34·25	14·27	105·89	47·15	39·44
Total digested	1570·10	1533·23	240·78	44·48	1222·54	25·43	36·87
Digested from basal food	967·48	944·13	230·27	43·18	656·54	14·14	23·35
Digested from tapioca flour	602·62	589·10	10·51	1·30	566·00	11·29	13·52
Digestion coefficients of tapioca flour, %	97·51	97·71	82·56	38·92	99·41	64·55	89·30
			Pig IX.				
Total consumed (as above)	1811·10	1734·79	275·03	58·75	1328·43	72·58	76·31
Total voided	236·47	197·67	36·00	15·53	101·89	44·25	38·80
Total digested	1574·63	1537·12	239·03	43·22	1226·54	28·33	37·51
Digested from basal food	980·05	953·78	232·11	42·99	665·50	13·18	26·27
Digested from tapioca flour	594·58	583·34	6·92	0·23	561·04	15·15	11·24
Digestion coefficients of tapioca flour, %	96·21	96·76	54·36	6·89	98·54	86·62	74·24

Digestibility of entire ration (digestion period 3).

Pig VIII	86·69	88·38	87·55	75·71	92·03	35·04	48·32
Pig IX	86·94	88·61	86·91	73·57	92·33	39·03	49·15

Comments on Table V.

The digestion coefficients of the *total* ration tested in period 3 display remarkably close agreement for both animals. The values indicate that the inclusion of tapioca flour led to a significant improvement of the already high digestibility of the fish meal, middlings and maize meal mixture, the digestion coefficient of the total organic matter (88·5 per cent.) in the ration containing tapioca flour being distinctly higher than the corresponding value (83·8 per cent.) for the basal mixture. The

improvement in the digestibility of the N-free extractives brought about by including tapioca flour in the ration is especially noteworthy.

These findings are substantiated by an inspection of the unusually high digestion coefficients of the total organic matter and the N-free extractives in the tapioca flour itself, namely, 97·2 and 99·0 per cent. respectively (mean values for both pigs). It is clear that the N-free extractives of tapioca flour are almost completely digestible, a result which is very striking, despite considerations of the favourable physical condition of the food and the presence of starch as the main form of carbohydrate. The significance of the values of the digestion coefficients of tapioca flour will be better appreciated by comparing them with corresponding values obtained in pig digestion trials of barley meal, maize meal and flaked maize. This comparison is shown in Table VI, together with a comparison of the percentages of total digestible organic matter in the dry matter of the feeding stuffs.

Table VI. *Digestibility of tapioca flour, barley meal, maize meal and flaked maize.*

	Digestion coefficient of total organic matter %	Total digestible organic matter (dry matter basis) %	Digestion coefficient of N-free extractives %	Digestible N-free extractives (dry matter basis) %
Tapioca flour	97·2	94·8	99·0	91·2
Barley meal (6)	81·7	79·9	88·7	69·3
Maize meal (8)	87·8	86·1	92·0	73·4
Flaked maize (8)	95·4	94·4	97·1	82·0

It will be noted from Table VI that tapioca flour is not only very distinctly superior to barley meal in respect of digestibility, but is also more digestible than maize meal. Indeed, the figures indicate that tapioca flour is slightly more digestible than flaked maize, which is one of the most digestible foods employed in the feeding of live-stock. Of the 94·8 parts of digestible organic matter in 100 parts of the dry matter in tapioca flour, 91·2 parts are in the form of digestible N-free extractives. Obviously, therefore, tapioca flour is to be regarded solely as a source of digestible carbohydrate in the rations of swine, its content of this constituent being distinctly higher than that of flaked maize and very much higher than that of barley meal (see Table VI). Of the feeding stuffs cited in this table, tapioca flour is the most unbalanced.

Since tapioca flour, barley meal, maize meal and flaked maize are all starch-rich feeding stuffs, some idea of their relative values for pig-feeding may be gained by comparing their contents of digestible organic

matter. It will be noted from Table VI that tapioca flour and flaked maize are about equal in this respect, and it may be concluded that, provided the ration contains sufficient protein- and mineral-rich food, tapioca flour should be able to replace flaked maize, lb. for lb., in the rations of swine. On the same line of reasoning, 1 lb. of tapioca flour should be able to replace $1\frac{1}{10}$ lb. of maize or $1\frac{1}{5}$ lb. of barley, provided, of course, that the various feeding stuffs have roughly the same moisture content. It may be concluded, therefore, that as a food for pigs, tapioca flour is of slightly better value than maize meal, and its extraordinary richness in digestible carbohydrate renders it especially suitable for inclusion in the rations during the fattening period. How this conclusion holds in actual farm practice will be made clear in the later section dealing with the large-scale farm feeding trials of tapioca flour.

Table VII. *Digestibility of sago pith meal (digestion period 1).*

	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extrac- tives gm.	Crude fibre gm.	Ash gm.
			Pig VIII.				
Fish meal	132·50	101·81	95·47	5·38	0·96	—	30·69
Middlings	432·65	412·19	89·43	21·68	269·02	32·06	20·46
Maize meal	619·57	610·34	74·60	27·94	485·31	22·49	9·23
Sago pith meal	608·44	584·47	11·80	2·68	539·75	30·24	23·97
Total consumed	1793·16	1708·81	271·30	57·68	1295·04	84·79	84·35
Total voided	409·20	347·72	78·25	14·46	178·03	76·98	61·48
Total digested	1383·96	1361·09	193·05	43·22	1117·01	7·81	22·87
Digested from basal food	960·98	937·93	227·82	42·86	653·25	14·00	23·05
Digested from sago pith meal	422·98	423·16	—	0·36	463·76	—	—
Digestion coefficients of sago pith meal, %	69·52	72·40	—	13·43	85·92	—	—
			Pig IX.				
Total consumed (as above)	1793·16	1708·81	271·30	57·68	1295·04	84·79	84·35
Total voided	385·94	328·48	72·00	12·93	171·55	72·00	57·46
Total digested	1407·22	1380·33	199·30	44·75	1123·49	12·79	26·89
Digested from basal food	973·44	947·51	229·63	42·67	662·16	13·05	25·93
Digested from sago pith meal	433·78	432·82	—	2·08	461·33	—	0·96
Digestion coefficients of sago pith meal, %	71·29	74·05	—	77·61	85·47	—	4·00

Digestibility of entire ration (digestion period 1).

Pig VIII	77·18	79·65	71·15	74·93	86·25	9·21	27·11
Pig IX	78·48	80·78	73·46	77·58	86·75	15·08	31·88

It will be seen from Table V that the digestion coefficients for the two animals in respect of protein, ether extract and fibre of the tapioca

flour displayed lack of agreement, despite the harmony shown by the corresponding values for the *total* protein, ether extract and fibre in the *entire* ration. This is scarcely surprising, however, since the amounts of these constituents in the tapioca flour were so insignificant as to render a reliable determination of their digestibility almost impossible. This lack of agreement was of no moment, in view of the fact, already established, that tapioca flour is included in rations solely on account of its richness in digestible carbohydrate, and that the necessary protein and fibre is supplied in the other ingredients of the ration. An artificial determination of the digestion coefficient of the protein in tapioca flour by the action of pepsin-HCl at 37° C. gave the value 60·7 per cent.

Comments on Table VII.

The satisfactory character of the trial in period 1 is indicated by the general agreement of the results, apart from those of the fibre constituent, for the two pigs in respect of the digestion coefficients of the *entire* experimental ration. The mean digestibility of the total organic matter in the ration (80·2 per cent.) is lower than the corresponding value (83·8 per cent.) for the basal mixture as tested in period 2, the inclusion of the sago pith meal having led, in particular, to a pronounced depression of the digestibility of the protein in the ration. These findings, considered in conjunction with the data in Table V, clearly suggest that sago pith meal is distinctly less digestible than tapioca flour, a conclusion which is confirmed by the comparative data recorded in Table VIII.

Table VIII. *Digestibility of sago pith meal, tapioca flour, barley meal and maize meal.*

	Digestion coefficients of total organic matter %	Total digestible organic matter (dry matter basis) %	Digestion coefficients of N-free extractives %	Digestible N-free extractives (dry matter basis) %
Sago pith meal	73·2	70·3*	85·7	76·0*
Tapioca flour	97·2	94·8	99·0	91·2
Barley meal(6)	81·7	79·9	88·7	69·3
Maize meal(8)	87·8	86·1	92·0	73·4

* For explanation of these apparently anomalous results for sago pith meal, see comments on this table.

It will be noted from Table VIII that the N-free extractives of sago pith meal are distinctly of lower digestibility than the corresponding fraction in both tapioca flour and maize meal, but only slightly less digestible than the N-free extractives of barley meal, the digestion

coefficients in this connection for sago pith meal and barley meal being 85·7 and 88·7 per cent. respectively. Owing to its higher content of N-free extractives, however, sago pith meal contains in its dry matter about 7 per cent. more digestible N-free extractives than barley meal.

The results for sago pith meal in Table VIII present an anomalous feature. Although this feeding stuff contains, on the basis of dry matter, 76·0 per cent. of digestible N-free extractives, yet every 100 parts of its dry matter contributes to the ration only 70·3 parts of digestible organic matter. The explanation of this abnormal result is apparent from a study of the data in Table VII. The inclusion of sago pith meal resulted in a pronounced depression of the ration as a whole, the depression, which was noted in an almost equal degree with both pigs, being particularly marked in the case of the protein constituent. Considering the case of pig VIII, the animal should have been able to digest about 228 gm. of protein from the basal mixture alone (*i.e.* from the mixture of fish meal, middlings and maize meal), if the digestibility of this mixture had been unaffected by the inclusion of the sago pith meal. Actually, however, the animal digested only 193 gm. of protein from the *entire* ration (*i.e.* from the basal food plus the added sago pith meal). The inclusion of sago pith meal, therefore, not only failed to augment the supply of digestible protein in the basal mixture, but actually reduced by 35 gm. the amount of digestible protein which should have been available from the mixture of fish meal, middlings and maize meal. It will also be noted that a similar effect was exerted on the digestion of the fibre in the ration.

It is not easy to assign a reason for the depressing influence of the sago pith meal on the extent of protein digestion. It could scarcely be ascribed to a big increase in the amount of "metabolic" nitrogenous material eliminated in the faeces, since the latter were of a perfectly normal character for both pigs throughout the feeding period and showed no evidence of the presence of mucous substances. It appeared more probable that the sago pith meal contained no digestible protein whatsoever, since digestion of the feeding stuff with pepsin-HCl at 37° C. failed to bring about solution of any of its nitrogenous constituents, the whole of the nitrogen of the food remaining in the insoluble residue from the digestion. A similar result was also obtained when the sago pith meal was incubated with trypsin under the requisite conditions. Indeed, it is doubtful whether the nitrogenous fraction of this feeding stuff is in the form of protein at all, since the protein colour reactions gave negative results on both the sago pith meal and on 10 per cent.

salt extracts of the food product. It may be concluded that the nitrogen of sago pith meal is present in an insoluble form other than protein, and that this feeding stuff is incapable of contributing digestible protein to the ration. Further, its inclusion in the pig's diet to the extent of about one-third of the total meal exerts a significant depression on the digestibility of the protein of the other foods contained in the ration.

The digestion coefficients obtained for the ether extract of the sago pith meal were discordant and untrustworthy (see Table VII), owing to the fact that of the total of 57·7 gm. of ether extract in the experimental ration, only 2·7 gm. came from the sago pith meal, this amount being too small to permit of reliable measurements.

If sago pith meal could be included in swine rations without causing a depression of the extent of utilisation of the protein in the accompanying feeding stuffs, then every 100 lb. of its dry matter would supply 76 lb. of digestible carbohydrate, not more than ⅓ lb. of digestible oil and no digestible protein, that is to say, a total of rather more than 76 lb. of digestible organic matter. In the case of barley meal[6], 100 lb. of dry matter would furnish about 69 lb. of digestible carbohydrate, 10 lb. of digestible protein and very small amounts of digestible oil and digestible fibre, totalling about 80 lb. of digestible organic matter. In pig rations containing a sufficiency of protein and minerals from other sources, sago pith meal should therefore have a value nearly equal to that of barley meal, provided that the proportion of sago pith meal in the ration is small enough to ensure that its depressing influence on protein digestibility is not very marked. What actually is the limiting amount of sago pith meal which may be fed without causing this depression is not clear from the present digestion trials, although the evidence of the farm feeding trials to be described in the second section of this paper warrants the belief that up to 20 per cent. of the total ration may be included with safety. If larger allowances of sago pith meal be given (as in digestion period 1, where one-third of the ration consisted of this feeding stuff), then its depressing influence on protein digestibility becomes significant, and it will have a definitely lower value than an equal weight of barley meal. Under these conditions, 100 lb. of its dry matter will supply only about 70 lb. of digestible organic matter compared with about 80 lb. from barley meal.

In respect of the data in Table IX, it is only necessary to comment on the finding that the mean daily live-weight increase in period 3 was smaller than that in period 2 and, in the case of pig IX, smaller than the gain in period 1, despite the fact that the ration in period 3 was

distinctly more digestible than the rations fed in the other periods. It should be kept in mind, however, that when period 3 was begun, the animals were about 2 stones heavier than at the beginning of period 2 and about 3 stones heavier than when the first period of feeding was begun. Since the weight of meal fed per day to the animals was almost the same in all three periods, it follows that as live-weight increased, a gradually increasing proportion of the digestible nutrients was being utilised for maintenance purposes, leaving a correspondingly smaller proportion for the purposes of live-weight gain. Viewed in this light, the live-weight data in Table IX are in accordance with what might have been anticipated.

Table IX. *Summary of nitrogen balances and live-weight changes in the pigs during the digestion trials.*

| Period | Pig | N consumed per day gm. | N voided per day | | | Mean daily nitrogen balance gm. | Mean daily live-weight increase lb. |
			Faeces gm.	Urine gm.	Total gm.		
1. Including sago pith meal	VIII	43·41	12·52	19·27	31·79	+11·62	0·80
	IX	43·41	11·52	16·42	27·94	+15·47	1·13
2. Basal food	VIII	62·91	7·68	39·96	47·64	+15·27	1·19
	IX	62·91	7·24	40·78	48·02	+14·89	1·31
3. Including tapioca flour	VIII	44·00	5·48	24·62	30·10	+13·90	1·00
	IX	44·00	5·76	23·98	29·74	+14·26	1·01

II. Farm trials.

Three separate feeding trials were carried out at the Animal Nutrition Piggeries on the University Howe Hill Farm in connection with the testing of the value of tapioca flour and sago pith meal in the feeding of the bacon pig.

Trial A (November 7, 1929 to February 20, 1930). A direct comparison was made between tapioca flour and sago pith meal, both feeding stuffs being included in the rations, from 80 lb. live-weight to slaughter, in partial replacement of barley meal.

Trial B (April 24, 1930 to September 2, 1930). Sago pith meal was tested in the rations of bacon pigs from 100 lb. live-weight to the date of slaughter.

Trial C (October 20, 1930 to February 12, 1931). This trial was substantially a repetition of trial B, the amount of sago pith meal in the ration, however, being restricted to 20 per cent. of the total ration, whereas in trial B, 40 per cent. of this product was included during the second half of the period of feeding. In addition, a third group of pigs

was included in trial C for the purpose of testing the efficiency of sago pith meal plus a small allowance of bran as a substitute for middlings.

Table X. *Survey of experimental rations employed in farm trials.*

Trial A.

	Group 1.			Group 2.	
	To 140 lb. live-weight %	140 lb. to slaughter %		To 140 lb. live-weight %	140 lb. to slaughter %
Barley meal	24	40	Barley meal	24	40
Tapioca flour	24	40	Sago pith meal	24	40
Middlings	40	13	Middlings	40	13
Extracted soya bean meal	10	5	Extracted soya bean meal	10	5
Minerals*	2	2	Minerals	2	2

Trial B.

	Group 3.			Group 4.	
	To 150 lb. live-weight. Parts by wt.	150 lb. to slaughter. Parts by wt.		To 150 lb. live-weight. Parts by wt.	150 lb. to slaughter. Parts by wt.
Barley meal	65	80	Barley meal	45	40
Middlings	25	15	Sago pith meal	20	40
Extracted soya bean meal	10	5	Middlings	25	15
Minerals*	2	2	Extracted soya bean meal	10	5
			Minerals*	2	2

   * Composed of ground limestone (6 parts), sterilised feeding bone flour (5 parts) and common salt (1 part).

Trial C.

	Group 5		Group 6		Group 7	
	To 140 lb. live-weight %	140 lb. to slaughter %	To 140 lb. live-weight %	140 lb. to slaughter %	To 140 lb. live-weight %	140 lb. to slaughter %
Barley meal	65	80	45	60	60	70
Sago pith meal	—	—	20	20	20	20
Bran	—	—	—	—	10	5
Middlings	25	15	25	15	—	—
White fish meal	10	5	10	5	10	5

The experimental animals in trials B and C were exclusively of the Large White breed. In trial A, however, a number of Large White-Large Black first cross-bred pigs were included in addition to the pure-bred Large White animals. The experimental groups consisted of ten pigs, except in one or two cases (see Table XI) where the exclusion of an animal became necessary, thus reducing the number of animals in such groups to nine. In dividing the animals into even experimental lots,

the factors of sex, live-weight, breeding, health and general condition were taken into account. The experimental period of feeding in the three trials was preceded by a pre-experimental period of adequate duration, in which the ration fed in common to all groups consisted of barley meal (45 parts), maize meal (15 parts), brown sharps (20 parts), bran (10 parts), fish meal (4 parts), meat and bone meal (4 parts), common salt (1 part) and ground limestone (1 part). The usual precautions were taken in respect of "worming" and inoculation against swine erysipelas.

All the experimental groups were kept under similar housing conditions in well-lit piggeries with concrete floors and dunging passages. Wheat straw was used as litter and changed as required. In the case of the winter trials A and C, thatched hurdles were laid across the tops of the pens to add to the comfort of the pigs. Weighing of the pigs during the course of the trial was carried out on the Thursday of each week at 8 a.m. For the purpose of the critical weighings at the beginning and end of the experimental period, however, the live-weights were taken on three consecutive days and the means of the three weighings taken to represent the true live-weights. The experimental rations were soaked in sufficient water to give the consistency of a thick paste and were fed in two meals during the day (7.30 a.m. and 4.30 p.m.). The size of the rations was adjusted to the amounts which the pigs could comfortably clear up in about 30 minutes. Fresh water was always available in all pens.

Table XI. *Results obtained in farm trials.*

Averages	Trial A		Trial B		Trial C		
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Initial live-weight (lb.)	77·3	81·1	100·6	109·0	102·4	100·8	99·4
Final live-weight (lb.)	180·4	158·2	198·7	185·5	188·7	187·5	186·5
Days in experimental period	77	77	56	56	63	63	63
Total live-weight gain (lb.)	103·1	77·1	98·1	76·5	86·3	86·7	87·1
Mean daily live-weight gain (lb.)	1·34	1·00	1·75	1·37	1·37	1·38	1·38
Meal per lb. live-weight gain (lb.)	4·03	4·69	3·82	4·34	4·64	4·76	4·72
Meal consumed per day (lb.)	5·40	4·69	6·68	5·94	6·35	6·56	6·51
No. of prime carcasses	8	9	5	9	9	9	5
No. of pigs in group	10	9	10	10	9	10	10

Comments on Table XI.

Trial A. The results in this trial were distinctly in favour of the ration containing 24, increasing to 40 per cent. of tapioca flour as against that containing corresponding amounts of sago pith meal. Over a period

of 77 days, the tapioca-fed animals averaged 1·34 lb. of live-weight increase per day, while the pigs receiving sago pith meal displayed a mean live-weight gain of only 1 lb. per day. Further, the ration containing tapioca flour was distinctly superior to the sago pith meal ration in respect of economy of food conversion, 1 lb. of live-weight increase requiring 4·69 lb. of meal in the latter case and only 4·03 lb. in the former. One reason for the quicker rate of live-weight increase in the group of pigs receiving tapioca flour was that these animals were able to consume a heavier ration (5·40 lb. of meal per day) than was the case with the pigs in the sago pith meal group (4·69 lb. of meal per day). This was a consequence of the greater bulkiness of the sago pith meal. Since it was noted that with increasing live-weight, the pigs in group 2 were able to deal more easily with the sago pith meal ration, it was decided to carry out a further test (trial B) of this feeding stuff against barley meal, restricting the comparison to the rates of live-weight gain in the period from 100 lb. to slaughter.

Trial B. The results of this trial in respect of rate of live-weight increase should not be compared with those of trial A, since the rations in the two trials were of very different composition and the weight changes were followed over different periods in the growth of the animals. In addition, trial A was carried out in winter, whereas the second trial was made during the summer months. The group 3 animals in trial B received a ration composed of barley meal, middlings, extracted soya bean meal and minerals. The ration of the group 4 pigs differed from the foregoing in one respect only, namely, that the barley meal was replaced in part by sago pith meal, the latter amounting to about 20 per cent. of the total ration over the period from 100 lb. to 150 lb. live-weight, and about 40 per cent. from 150 lb. to slaughter weight.

The pigs in group 3 made quicker gains of live-weight than those in group 4, the mean daily gains over the feeding period being 1·75 lb. and 1·37 lb. respectively. The partial replacement of barley meal by sago pith meal led to a decrease in both the economy of food conversion (4·34 lb. of meal per lb. of live-weight increase as against 3·82 lb. in the case of the barley meal control ration) and the amount of meal the pigs were able to consume per day (5·94 lb. per day in group 4 as against 6·68 lb. in the control group). Since the pigs in group 4 received a distinct check in growth rate at the stage when the proportion of sago pith meal was increased to 40 per cent. of the ration, it was deemed advisable to carry out a third test (trial C) in which the amount of sago pith meal was kept throughout at the low level of 20 per cent. of the

ration. As in trial B, the comparison with the control ration was restricted to the period of feeding between 100 lb. live-weight and slaughter.

Trial C. The ration of the control animals (group 5) consisted of barley meal, middlings and fish meal, the barley amounting to 65 per cent., increasing to 80 per cent. of the total ration (see Table X). The ration of the experimental animals in group 6 differed from the control ration in having 20 parts of the barley meal replaced by sago pith meal. The results in Table XI reveal no significant differences in the rate of gain of live-weight of the animals in the two groups, the number of pounds of food required per lb. of live-weight gain and the amount of meal consumed per day. Obviously, under the conditions obtaining in trial C, where the amount of sago pith meal was restricted to 20 per cent. of the ration, the value of this food was equal to that of barley meal.

A third group of pigs (group 7) was included in trial C. These animals were given a ration in which the middlings of the control ration were replaced by sago pith meal plus a small allowance of bran. Up to 140 lb. live-weight, the 65 parts of barley plus 25 parts of middlings in the control ration were replaced by 60 parts of barley, 20 parts of sago pith meal and 10 parts of bran, the fibre in the two rations being about 4·5 and 4·6 per cent. respectively. From 140 lb. to slaughter, 80 parts of barley and 15 parts of middlings were replaced by 70 parts of barley, 20 parts of sago pith meal and 5 parts of bran, the fibre now representing about 4·5 per cent. in both rations. It will be seen from the results in Table XI that under these conditions of replacement, the ration containing sago pith meal and bran gave results equal to those produced by the control ration, there being no significant differences between the results for groups 5, 6 and 7 in respect of rate of gain of live-weight, amounts of meal consumed and economies of food conversion.

The following conclusions may be drawn from the results of the farm trials:

(1) Sago pith meal as a substitute for barley meal may be employed in the rations of bacon pigs from 100 lb. live-weight to slaughter up to 20 per cent. of the ration without causing any depression of the rate of live-weight gain, economy of food conversion or the amount of meal consumed. If the replacement is increased to about 40 per cent. of the ration, the effect exerted by sago pith meal is distinctly less than that of barley meal. This is evidenced by a smaller consumption of food by the animals, a lower rate of live-weight increase and a higher requirement of food per lb. of live-weight gain. These findings are in agreement

with the conclusions arrived at on the basis of the digestion trials which were described in the first section of this communication.

(2) Sago pith meal plus a small allowance of bran is an efficient substitute for middlings in the ration of bacon pigs, provided the sago pith meal used for this purpose amounts to no more than 20 per cent. of the total ration.

(3) Tapioca flour is superior to sago pith meal as a carbohydrate food for pigs and is at least equal to barley meal. The findings of the digestion trials place tapioca flour on an equality with maize, or even flaked maize, in respect of feeding value, a conclusion which is substantiated by the results of feeding trials with pigs carried out by Fullerton[1] and Howie[2], who concluded that tapioca flour may replace maize meal up to at least 25 per cent. of the ration. In the present feeding trials, it will be noted that tapioca flour formed as much as 40 per cent. of the ration in the second half of the feeding period. Further confirmation of these results is forthcoming from the recent pig-feeding tests of Hansson and Bengtsson[3] in Sweden. These workers concluded that tapioca flour is at least equal in feeding value to barley or maize, and that, provided the ration contains the requisite supplement of mineral- and protein-rich food, tapioca flour may be fed to the extent of about 3 lb. per head per day in the final period of fattening.

<div align="center">SLAUGHTER RESULTS.</div>

Trial A. It was noted that tapioca flour exerts a distinctly favourable influence on the colour and texture of the fat and the quality of the bacon, an observation which is amply confirmed by the work of earlier investigators[1, 2, 3]. Of the 10 carcasses examined, 8 were graded by the bacon factory as "prime"; *i.e.* the animals were lean and their carcasses of the desired quality and conformation for the production of first class bacon. The mean carcass percentage for this group, based on the factory live-weight, was 78·6 per cent.

The animals in group 2, which received sago pith meal up to 40 per cent. of the ration, were lean and of excellent conformation for bacon production. The back-fat measurements at the thickest point were 19 per cent. less than those of the pigs in group 1 which received tapioca flour in the ration. The mean carcass percentage was 76·1 per cent. It is especially noteworthy that all the carcasses in this group were graded as "prime."

Trial B. Only 5 out of the 10 carcasses from the control group were graded as "prime," the mean carcass percentage being 76 per cent. On

the other hand, the beneficial influence of sago pith meal on quality and conformation was again noted, 9 out of the 10 carcasses from group 4 being graded as "prime." The mean carcass percentage for this group, however, was low, namely, 73·2 per cent.

Trial C. The mean carcass percentage of the control group receiving barley meal, middlings and fish meal was 76·2 per cent., and all the 9 carcasses were graded as "prime." In the case of the group receiving sago pith meal in replacement of barley meal up to 20 per cent. of the ration, 9 out of 10 carcasses were graded as "prime," and the mean carcass percentage was 75·4 per cent., this being a somewhat higher value than for the pigs in group 4 (trial B) which received sago pith meal up to 40 per cent. of the ration.

It is interesting to note that when sago pith meal and bran were used as a substitute for middlings, the favourable influence of the sago pith meal on quality and conformation appeared not to have been in evidence, since only 5 out of the 10 carcasses in group 7 were graded as "prime." The mean carcass percentage for this group was 75·8 per cent.

GENERAL CONCLUSIONS.

1. It has been shown that tapioca flour is one of the most digestible feeding stuffs employed in swine husbandry. The finding of earlier workers that tapioca flour is able to replace barley or maize in the rations of pigs is substantiated by the results of the digestion trials and farm trials which have been described in this paper. In the feeding tests conducted by Fullerton[1] and Howie[2], the experimental animals were given rations which contained up to 25 per cent. of tapioca flour. In the present feeding trials, the ration of the bacon pigs from 140 lb. live-weight to slaughter included 40 per cent. of this product. Hansson and Bengtsson[3], in recent Swedish feeding experiments, found that, provided the ration of bacon pigs contains the requisite protein- and mineral-rich supplements, tapioca flour may be fed to the extent of about 3 lb. per head per day in the final finishing period. This actually was the amount of tapioca flour which the animals were receiving in the present trial during the three weeks prior to slaughter.

2. The results of the present trials confirm the conclusion of earlier investigators that tapioca flour exerts a distinctly favourable influence on the colour and texture of the carcass fat and on the quality of the bacon.

3. Sago pith meal, a new carbohydrate food coming from Malaya, may be introduced into the rations of bacon pigs, as a substitute for

barley meal, up to 20 per cent. of the ration without depressing the rate of gain of live-weight. It is more suitable for pigs of 100 lb. live-weight or more than for younger animals. If the replacement is increased to 40 per cent. of the ration, the effect exerted by sago pith meal is distinctly less than that of barley meal. This is evidenced by a depression of the amount of food consumed by the animals, a lowering of the rate of live-weight increase and a raising of the amount of food required per lb. of live-weight increase. The findings of the digestion trials suggest that this apparent lowering of the efficiency of sago pith meal as the amount in the ration is increased beyond 20 per cent. is due to a depressing influence which it exerts on the digestibility of the other foods in the diet, in particular, on the digestibility of the protein in the rest of the ration. Sago pith meal itself appears to contain no digestible protein.

4. Sago pith meal plus a small allowance of bran has been found to be an efficient substitute for middlings in the rations of bacon pigs of more than 100 lb. live-weight, provided the sago pith meal amounts to no more than 20 per cent. of the ration.

5. Sago pith meal has a favourable effect on quality and conformation. Out of a total of 29 carcasses from pigs which had been given rations containing sago pith meal in partial replacement of barley meal, 27 were graded as "prime." On the other hand, however, only 5 "prime" carcasses were obtained from a group of 10 pigs which had received sago pith meal and bran as a substitute for middlings.

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STUDIES IN THE YIELD OF TEA.

I. THE EXPERIMENTAL ERRORS OF FIELD EXPERIMENTS WITH TEA.

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(With One Text-figure.)

THE increased interest in field experimental technique that has been noticeable in recent years has a two-fold origin. As the broad generalisations with regard to crop management, such as emerged from the classical experiments of Lawes and Gilbert, became incorporated into current agricultural practice, attention was turned to problems of greater intricacy. In the spheres of manurial practice and of varietal differences there was room for experimentation involving comparisons that were less spectacular in their results than the earlier trials, but which were nevertheless of considerable economic importance to agricultural industry. When, however, these comparisons were attempted it was found that in many cases the experimental errors involved in carrying out the trials in the field were so great that reliable results as to the superiority of some of the treatments under consideration could not be obtained unless a reduction in error could be effected. At the same time the diversity of crops investigated increased enormously, and experimenters discovered that especially where tropical crops were concerned, techniques evolved in temperate regions could not be profitably applied under new conditions without modification.

The dual attempt to increase experimental accuracy in temperate crops and to apply the resulting improvements elsewhere has met with greater success in the former case than in the latter. From the time of Mercer and Hall's pioneer investigation on the errors of wheat and mangolds [10] continuous progress has been made in the design of experiments and in the application of statistical methods to the study of error, so that at the present time the errors to be expected from field experiments on temperate annual crops are rather precisely known. The Rothamsted experiments from 1925–9 [12, 13] show that stability in error for a variety of crops is attainable.

Tropical crop experiments as a whole lack a corresponding degree of precision. In the first place, the fundamental difficulties are greater, and, in the second place, the cultivation of tropical produce has only recently become sufficiently intensive to make small yield increments important.

The tea crop provides a good example of this latter fact. The mean yield per acre of tea in Ceylon rose from 350 lb.[1] to 500 lb. during the first quarter of a century, and consequently tea cultivation has now reached the stage of small yield increments necessitating accurate experimentation.

When the present investigation was started the literature of tea experiments in Java, India and Ceylon contained no reference to experimental error. Prillwitz[11] has recently published a summary of the relationship between plot size and error to which reference will be made again later, but in Java plots have ranged from small fractions to several acres in extent. In India, it has been the practice to employ plots as small as a fiftieth of an acre with a replication as low as threefold. None of these experiments is suitable for strict statistical analysis, but a recent Indian experiment covering four years' growth gives an approximate error value of 8·25 per cent. for differences between triplicate means[2, 3].

Difficulties in tea experiments occur at many points and, therefore, before considering these and defining the scope of the enquiry, an outline of the general characteristics of tea culture is necessary for the better understanding of points that will occur later.

CHARACTERISTICS OF TEA CULTURE.

Tea (*Thea sinensis*) is a tree which, if left to grow naturally, frequently reaches a height of from 20 to 30 ft. It normally produces flowers and sets seed, and under these conditions leaf growth is relatively small. As a crop cultivated for tea manufacture, growth is severely controlled, the main stem being pruned back a few inches above ground level after the first few years of normal growth, with a view to inducing the plant to put out laterals and assume the bush habit. Following the primary pruning or centring, fresh growth is allowed till the bushes have filled out and attained an over-all width of 20–24 in. measured at a point 18–20 in. above ground. They are then pruned back to a height of about 20 in. and taken into bearing. Hereafter at regular intervals, varying from 18 months to over 3 years according to altitude and locality, the

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[1] This figure represents made tea; in terms of green leaf the values are approximately fourfold.

bush is pruned regularly, the new cuts being made 2-3 in. above the old ones until the bush height becomes too great and necessitates down-pruning. In practice it is found almost impossible to treat each shoot strictly on its merits, but the weaker centre shoots are taken out, leaving strong wood to form the frame. Time is allowed for recovery from pruning and then the young succulent shoots are broken off or tipped so as to produce a flat or slightly convex upper surface or plucking table. At this stage the bush can be plucked for the manufacture of the young leaves or flush into normal tea.

Whenever a bush is tipped or plucked, the buds in the axils of the leaves below are stimulated into growth and new shoots are put out from the growing points. The bud scales drop off with the exception of one or two that may develop into leaves of moderate dimensions. Gadd(5) suggests that the so-called fish leaves which differ in both shape and size from normal leaves are in all probability developed scale leaves. The new shoot is allowed to elongate until at least three normal leaves have expanded above the fish leaf. It is this new growth that is termed the flush; from this alone leaves are plucked. Two leaves and the terminal bud are customarily plucked, leaving at least one normal leaf above the fish leaf still on the bush. In this way not only is the retention of a leaf that contains an unexpanded axillary bud assured for the next growth period, but an addition is made to the number of new normal foliage leaves for the support of the growing bush. If by chance the shoot is plucked down to the fish leaf, the probability of that shoot producing a strong healthy growth is decreased. The length of time necessary for the production of new flush varies according to seasonal climatic conditions and altitude, being as short as 7 days at low altitudes and as long as 16–18 days at 6000 ft.

From this short description of the growth and management of tea, the special difficulties of field experiments can be easily visualised. In the first place, the available population for experiments of moderate dimensions is much smaller than in ordinary farm crops and the sample is less representative. A normal stand of tea represents approximately 3000 bushes to the acre. There are numerous opportunities afforded to the bush in the process of growth for developing a marked individuality. Bad centring, pruning and bad plucking all affect the factors of which the flush removed may be regarded as the integration. Of these factors, plucking is the hardest to control. One aims at a definite standard of plucking, but the human material available is not of a high order. Another cause conducive to high error is the very variable yield obtained on the

different plucking rounds. During a year's plucking, embodying the complete seasonal climatic cycle, the yields showed extremes of 11 lb. and 55 lb. of dry matter per acre with a mean of 27 lb. Finally, in addition to bush variation there is the inevitable soil heterogeneity. Tea is pre-eminently a hill-country crop in Ceylon; level areas are almost unknown and even on regular slopes, soil erosion, variable soil depth and drainage are only too often encountered. These are the fundamental difficulties; others are dealt with in more detail in their appropriate context.

AIM AND SCOPE OF THE INVESTIGATION.

As a result of the considerations set out in the preceding section, it seemed to the writer that, before undertaking experiments of a definitely agricultural nature, it was essential to study the tea crop and its management in their more fundamental aspects. The work which follows may be conveniently divided into five sections as follows:

(1) General methods of plot management.

(2) The influence on error of plot size and arrangement.

(3) The seasonal and plucking variation and their effect on experimental accuracy.

(4) The possibility and value of intermittent experimental plucking.

(5) The utility of previous crop records as a basis for forecasting and compensating yield and error determinations.

(1) GENERAL METHODS OF PLOT MANAGEMENT.

In pursuance of these aims a uniformity trial was projected. At the time the Institute possessed no estate of its own, but an area of two acres was placed at its disposal within a short distance of the Institute's temporary headquarters. The field in question was in full bearing and stood at an elevation of 6300 ft. above sea-level. The jat or type was, in common with much up-country tea, a so-called hybrid. The stand was even, though small differences in type between one bush and another were plainly visible, and the area was devoid of any other crop in the nature of ground cover or shade trees. The prevalent practice of clean weeding was observed. As far as the records go, the tea was approximately 46 years old at the start of the experiment. The normal pruning cycle of this field is 3 years and at the time when operations were begun the field was 15 months out of pruning. Whilst the disadvantage of not securing data over a complete pruning cycle was recognised, no alternative site was available, and it was considered that the yields from the remaining

portion of the pruning cycle would give useful and adequate indications on all points required. The plucking extended from April 20, 1928, to December 10, 1929, during which time 42 plucking rounds were recorded. During this time the field was subjected to the normal estate routine in
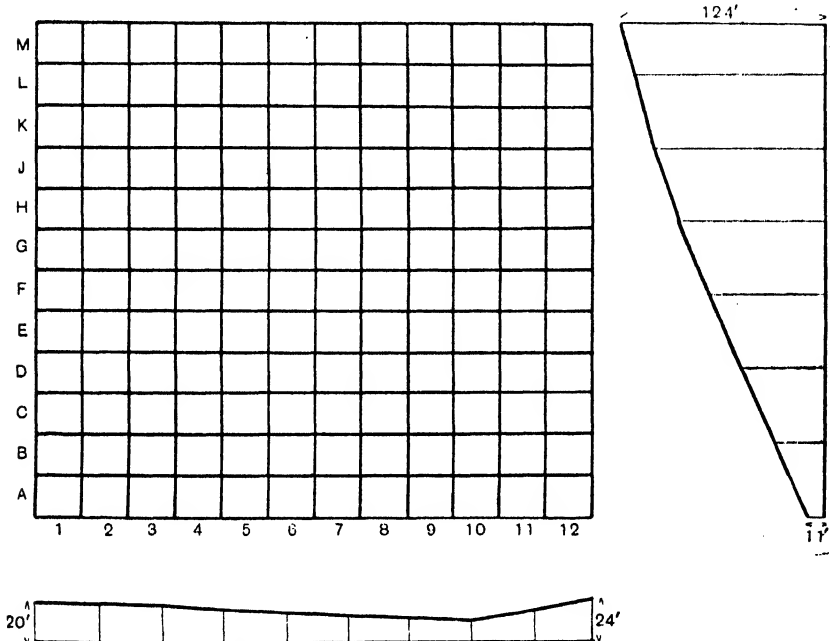
Fig. 1. Plan of uniformity trial and gradients.

all respects. During the period of the experiment the area was twice manured with a mixture supplying the following amounts of nutrient per acre:

	Nitrogen	Phosphoric acid	Potash
August, 1928	42 lb.	27·5 lb.	29·3 lb.
August, 1929	28 ,,	18·4 ,,	19·5 ,,

The area was divided into 144 plots of 1/72 acre, and each plot contained 6 rows of bushes; the appropriate area was obtained by measuring up the rows. Such a method frequently involved the division of a bush between two plots, but the boundary wire, placed a little higher than the plucking table between plots, adequately defined the plot limits. Each plot contained approximately 42 bushes. The general layout and contour of the area are shown in Fig. 1.

The programme of plucking that was adopted throughout was to

commence with row A. Each plot in the row was plucked by a separate individual into a registered basket assigned to that plot. On completion the plucker moved to the corresponding plot in row B, again plucking into a registered basket, and so on up the slope.

The method, besides minimising mistakes, contributed distinct advantages to the subsequent examination of the yield data. The same plucking team was employed on the same plots throughout. All weighings were made on the spot by the Institute staff.

Basis of yield determinations.

Tea, in Ceylon, is plucked continuously throughout the pruning cycle and some 50 per cent. of the plucked leaf comes to the weighing base carrying surface moisture from rain or dew. Fresh weight yields are thus not merely incomparable from plucking to plucking, but frequently parts of a single day's plucking are affected. The incidence of rain during the 24 hours of a day is such that even over a period there can be no balancing out of these effects. Consequently, all yields are expressed in terms of dry matter at the temperature of boiling water (94° C. at this altitude).

The dry weights were determined on the basis of samples taken as follows. Each plot in the individual lettered row was plucked, within fine limits, simultaneously. After weighing, a sample was taken from each basket and the 12 samples thus obtained bulked and mixed. From the composite sample, quadruplicate dry matter samples were taken, and from the mean moisture content of these, the dry matter yields for the whole row were calculated plot by plot. In all, therefore, 12 series of quadruplicate samples were drawn. The adequacy of this sampling was tested by examining the data collected under diverse climatic conditions. The total variation of the 48 samples of any individual plucking can be analysed into a portion representing differences between mean moisture content of samples collected at different times (inter-set), and a residue representing differences between samples drawn at the same time (intra-set). Since there were 12 sets of 4 samples each, the analysis of variance is of the form:

Variance	Degrees of freedom
Inter-set	11
Intra-set	36
Total	47

The intra-set variance provides a measure of random error applicable to the whole data. The results of this examination are given in the following table:

Table I. *Errors of fresh weight/dry weight for samples under varying conditions.*

Condition	Range	General mean	s.e.* (mean of 4)	s.e. %
No rain	3·79–4·16	3·93	0·0216	0·55
Rain latterly	3·76–4·75	4·14	0·0253	0·61
Continuous rain	4·62–5·24	5·06	0·1276	2·52

* S.E. = Standard error.

The difference between the extreme mean ratios represents an apparent increase in yield due to the adherent moisture yield of nearly 30 per cent. We are chiefly concerned with the highest ratio under conditions of heavy rain. It will be shown later that the standard error of the yield of single parallel plots is in the neighbourhood of 16 per cent. The addition to this of the largest dry matter error augments it by only 0·19 per cent., so that the sampling errors inherent in dry matter determinations are negligible. A minor advantage of using dry weights is that these figures correspond roughly with the outturn of made tea.

(2) The influence of plot size on error.

Soil heterogeneity and the corresponding variability of crop density and vigour are seldom of a purely random nature: plots nearly adjacent tend to behave alike and exhibit greater similarity than plots randomly chosen. Mercer and Hall (*loc. cit.*) showed this by building up plots from smaller units chosen, first of all, at random, and secondly, in contiguous positions, the latter giving the smaller error. Harris[6] provided a more exact expression of this in his coefficient of heterogeneity, and Maskell[9] confirmed Mercer and Hall's conclusions more precisely by applying Harris's criterion to their results.

Fisher[4] has shown that advantage can be taken of this correlation between yields of adjacent areas to eliminate those elements of soil heterogeneity which affect all treatment comparisons equally, leaving a reduced random variance from which to derive errors employed in testing the significance of different treatment responses. Designs which incorporate these principles have been highly successful on annual crops in England[13], but they have received little attention in tropical crop experiments, and their success is still debatable. Lord's results on paddy[7] and on rubber[8] give much smaller gains from the elimination of positional variance common to all treatment comparisons, than are usual with temperate crops. Beckett[1] inclines to the view that on permanent crops the heterogeneity of the material is so great as to obscure any difference due to treatment, whatever the system of plot layout. The

validity of this conclusion, as far as the present data is concerned, may be tested by making a comparison between the error results obtained on the lines of Fisher's method (in which positional variance is eliminated) and those obtained without regard to such elimination.

The latin square was chosen as it is the type in which positional variance can be most successfully studied. The whole population of 144 yields is divisible into four such squares which are dealt with in turn. The arrangement and total yields for the whole period are tabulated in Appendix A.

(a) 12×12 *latin square.*

The analysis of variance is given in Table II.

Hypothetical numbered treatments have been assigned to each plot of the uniformity trial in order that the analysis may correspond with that of actual experiments in which treatment comparisons are involved. In the analysis this hypothetical treatment variance is shown separately.

The mean square shows that the largest individual component of variance is attributable to rows. In terms of sums of squares it amounts to 28 per cent. of the whole. The other positional variance due to columns is seen to be much smaller. The mean square for the hypothetical treatments exceeds that for parallels. Since both are really independent estimates of random variance, they should be statistically equivalent.

Table II. *Analysis of variance for* 12×12 *latin square. Mean* 972·574.

Variance due to	Degrees of freedom	Sums of squares		Mean square
Treatment	11	349123		· 31738
Parallels	110	2559345		23267
	121		2908468	24037
Rows	11		1293136	117558
Columns	11		335336	30485
Total	143		4536940	

	Parallels	Parallels + treatment	Total
s.e.	152·54	155·04	178·12
s.e. %	15·68	15·94	18·31

Tests of significance.

	Z calculated	Z significant, $P = 0.05$
Treatment v. Parallels	0·1553	0·3093
Rows v. Parallels (new)	**0·7937**	0·3071
Columns v. Parallels (new)	0·1188	0·3071
Total v. Parallels (new)	0·1388	0·1452

Precise comparisons of variance are made in the second portion of the table by means of the statistic Z, half the difference of the $\log_e$ mean

square, for the pairs of values compared. The significant values for a 1 in 20 probability that the values are due to chance are taken from the published tables of Z (Fisher, *loc. cit.*) or calculated by the approximate methods given there in accordance with the degrees of freedom available as shown in the variance analysis. The treatment and parallel variances, being derived from very different degrees of freedom, are subject to very different standard deviations. The Z comparison, which takes this fact into account, shows that the apparent superiority of the treatment variances is not real. The fact that these two estimates of parallel variance do not differ essentially is evidence for the satisfactory randomisation of the latin square; it is justifiable, therefore, to amalgamate the two sources and to calculate a new mean square on the basis of the increased number of degrees of freedom. This is referred to as either Parallels + Treatment or Parallels (new). The other variances are tested against this latter value. That for rows is quite definitely significant and that for columns definitely not so. The significant row variance establishes a reasonable probability that the difference in row values is due to a real fertility slope. The insignificance of the column variance is of particular interest. The plucking routine described earlier in the paper allows each row to be plucked by identical pluckers, and therefore introduces no plucking error into the estimated row variance. The opposite holds for the columns, each column being plucked by a different individual; column variance is therefore a measure of soil heterogeneity plus plucking individuality. Unless one supposes that fortuitous circumstances have caused plucking individuality to be counterbalanced by soil and bush differences, the results presuppose a reasonable standardisation of plucking on the experimental plots as a whole. If this uniformity is confirmed in subsequent examination of the data, it removes one serious technical difficulty to which experiments with crops similar to tea are particularly liable. The latin square arrangement eliminates the possible effect of such plucking individuality, where present, both from mean estimates and error estimates; but with other arrangements on large experiments its elimination might prove difficult in view of the fact that all the experiment must be plucked on the same day.

When the comparison between the total and parallel variance is made on the same principle, the total variance is only probably greater than the parallel estimate: the plot unit has been too small to take advantage to any great extent of definite fertility slopes, and the success of the method is only moderate.

(b) 6 × 6 *latin square.*

Table III. *Analysis of variance. Mean* 3890·29.

Variance due to	Degrees of freedom	Sums of squares	Mean square
Treatment	5	223129	44626
Parallels	20	3494135	174707
	25	3717264	148691
Rows	5	2883751	576750
Columns	5	912721	182544
Total	35	7513736	

	Parallels	Parallels + treatment	Total
s.e.	417·98	385·60	458·37
s.e. %	10·74	9·91	11·78

Tests of significance.

	Z calculated	Z significant, P = 0·05
Treatment v. Parallels	−0·6824	0·7584
Rows v. Parallels (new)	**0·6777**	0·4783
Columns v. Parallels (new)	0·1025	0·4783
Total v. Parallels (new)	0·1728	0·3180

The 6 × 6 square is obtained by amalgamating the primary plot yields in units of four, each new plot being formed from A 1, A 2, B 1, B 2, and so on for the whole series (Appendix B). Table III gives the analysis as before.

The row variance is again significant, thus confirming the fertility slope, whilst, even with pluckers amalgamated in groups of two, no significant column variation is obtained. The treatment variance is sub-normal, but not significantly so. The parallel variance is thus greater than parallels + treatment, illustrating the point that where a definite attempt is made to allocate treatments so as to give equivalent values, the final error determination is augmented and not diminished as is sometimes supposed.

Total variance is again of the same order as the parallel variance.

(c) 4 × 4 *latin square.*

In this case the unit is of the form A 1-3, B 1-3, C 1-3 (Appendix C). The indications given by this analysis follow the same broad outline as those of its predecessors.

Row variance is significant and treatment variance, though sub-normal, is not significantly so. The reduced numbers of degrees of freedom make all comparisons less delicate.

Table IV. *Analysis of variance. Mean* 8753·16.

Variance due to	Degrees of freedom	Sums of squares		Mean square
Treatment	3	584679		194893
Parallels	6	3498596		583099
	9		4083275	453697
Rows	3		5645447	1881816
Columns	3		1005016	335005
Total	15		10733738	715583

	Parallels	Parallels + treatment	Total
S.E.	763·61	673·57	845·92
S.E. %	8·72	7·70	9·66

Tests of significance.

	Z calculated	Z significant, P = 0·05
Treatment v. Parallels	− 0·5480	1·0953
Rows v. Parallels (new)	**0·7113**	0·6757
Columns v. Parallels (new)	− 0·1517	1·0880
Total v. Parallels (new)	0·2278	0·5497

(d) 3 × 3 *latin square.*

Table V. *Analysis of variance. Mean* 15561·18.

Variance due to	Degrees of freedom	Sums of squares		Mean square
Treatment	2	38452		19226
Parallels	2	272656		136328
	4		311108	77777
Rows	2		6443238	3221619
Columns	2		2981468	1490734
Total	8		9735814	1216977

	Parallels	Parallels + treatment	Total
S.E.	369·23	278·89	1103·17
S.E. %	2·37	1·79	7·09

Tests of significance.

	Z calculated	Z significant, P = 0·05
Treatment v. Parallels	− 0·9794	1·4722
Rows v. Parallels (new)	**1·8619**	0·9690
Columns v. Parallels (new)	**1·4766**	0·9690
Total v. Parallels (new)	**1·3751**	0·8993

The plot unit is of 16 plots, each one being a 4 × 4 square of the primary yields (Appendix D).

The data for this analysis assume a different aspect. The arrangement has been markedly favourable, since not only do rows and columns give a significant variance, but the parallels are definitely less than the un-

analysed data. It must be borne in mind, however, that this arrangement is much more rigid than the others, and the element of randomness and the degrees of freedom are much reduced. In the ordinary course of events, such a small square, unless itself replicated, could not be regarded as satisfactory.

From the foregoing analyses a certain amount of information can be obtained as to suitable plot size. Whilst there are disadvantages in confining attention to these latin square examples only, one important advantage is gained, that of studying size relationships without the interfering influence of plot shape. To interpolate other plot sizes would have involved a much less complete elimination of positional variance, and in this actual trial the results would have depended largely on the shape of the plots. Apart from edge effects, the best plot shape is that which allows of a maximum elimination of positional variance; this will vary from situation to situation according to the fertility gradient of the land and the type of experimental arrangement contemplated.

The standard error per cent. shows an appreciable fall for 4-unit plots, a much smaller diminution for 9 units and another rapid fall to 16 units. Reasons for placing less reliance on the last result have been given, and for practical purposes the region of interest lies between the 4-unit and the 9-unit plots.

The Z comparison (difference of $\log_e$ s.e.) substantiates the drop in error for the first amalgamation but not for the second, the figures being:

	Z calculated	Z significant, $P=0.05$
12×12 v. 6×6	**0.4754**	Between 0.2685 and 0.3376
6×6 v. 4×4	0.2523	0.5320

The reduction in degrees of freedom makes a delicate comparison impossible. Some additional information is available from a consideration of the behaviour of total variance. This behaves in the same way, and further, when the 3×3 square is considered, shows that the parallel variance of the latter achieves a low value almost entirely by means of the favourable coincidence of positional variance.

	Z calculated	Z significant, $P=0.05$
12×12 v. 6×6	**0.4409**	Less than 0.3101
6×6 v. 4×4	0.1986	Between 0.3628 and 0.4138
4×4 v. 3×3	0.5079	0.5840

The safest inference to draw from these results is that the lowest plot size advisable is a 4-unit plot, *i.e.* $\frac{1}{18}$ acre, but that, if land is available, a saving in labour can be made by increasing this size to a further

limit of 9 units. In actual practice, if the latin square were used, a plot size between these two limits might safely be chosen according to the number of treatments that it was desired to accommodate on a given area. The actual standard errors of the mean for a fourfold unit with sixfold replication, and for a ninefold unit with a fourfold replication are 4·05 and 3·89 respectively. On the whole, the analyses support Beckett's contention (*loc. cit.*) that, owing to heterogeneity, experimentation with permanent crops raised from seedlings is at a disadvantage compared with that on annual crops, in that the removal of positional variance in the former case is relatively ineffective in reducing error.

The limits of plot size agree satisfactorily with those of Prillwitz (*loc. cit.*) who found a maximal error reduction at $\frac{1}{20}$ acre, but since no details of method or number of plots employed are given by this worker, exact comparisons of variance reduction are not possible. Prillwitz, however, gives a much smaller error per plot than we have found. The comparison is:

	Plot size	% s.e. of one plot
Java	$\frac{1}{20}$ acre	5·9
Ceylon	$\frac{1}{18}$,,	9·9

Without further details it is impossible to say whether Prillwitz was working under more uniform conditions of soil and jat than obtain here. Despite these figures, however, Prillwitz recommends plot sizes as high as $\frac{1}{4}$ acre.

This section may be summarised by recording that the elimination of positional variance is much less successful on tea than on annual crops, which phenomenon has also been noted for other tropical experiments. Plucking individuality has not proved serious over the experiment as a whole, whilst plots of $\frac{1}{18}$ acre have given results involving the greatest reduction in error ascertainable with certainty.

(3) Seasonal and plucking variation.

Where a crop is harvested regularly at short intervals, it is possible to obtain data on seasonal response, and to follow the factors at work in the process of integration. Examples of this which are likely to occur are the possible difference in yield curves produced by different qualitative sources of the same plant nutrient, and the varying behaviour over a season of yield curves where one plant nutrient is working in the presence or absence of a second. The value of the data collected in these connections will naturally depend on the errors of these yield curves,

Possibilities of plucking individuality here assume enhanced importance, and, although it has been shown that over a period a satisfactory standardisation of plucking has been reached, the analysis must in this connection be extended to the consideration of individual occasions. The data here presented provide an opportunity for such an analysis and for the study of the degree of correlation which exists between the various groups of factors separable from the total data. Table VI gives the analysis for the 42 pluckings of a 4 × 4 latin square.

The 671 available degrees of freedom have been partitioned out to show the primary variation of treatment, positions, parallels and occasion, and the differential effects of occasion on each of the others. By way of explanation of the method of analysis of differential effects, that due to treatment and occasion is considered in detail.

Considered as four treatments, carried out on the 42 occasions, the partition of degrees of freedom is as follows:

		Degrees of freedom
Within occasion: Treatment	...	3
	Treatment × Occasions	123
		126
Between occasions	... ...	41
	Total	167

The sum of squares of deviations appropriate to the 126 degrees of freedom within occasions is derived from the total of the treatment sums of squares on the 42 individual occasions (42 × 3 degrees of freedom): the difference between this figure and the sum of squares for treatment without respect to occasion, gives the differential value required. The same process for rows, columns and parallels provides the corresponding occasion differential sum of squares for those groups. In the table, the whole analysis reduced to a single plot basis is expressed in quarter pounds. The first four items correspond to the variances considered in Table IV.

Table VI.

Variance due to	Degrees of freedom	Sum of squares	Mean square
Treatment	3	139·2	46·40
Rows	3	1344·2	446·07
Columns	3	239·3	79·77
Parallels	6	833·0	138·83
Occasion	41	47958·6	1169·72
Occasion ⎧ Treatment	123	625·0	5·08
⎨ Rows	123	2142·4	17·42
⎩ Columns	123	2849·4	23·17
Parallels	246	1104·1	44·88
Total	671	57235·2	

The variation in yield performance from occasion to occasion is here demonstrated to be overwhelmingly the greatest value obtained, accounting as it does for over 80 per cent. of the sums of squares with only some 6 per cent. of the degrees of freedom. The significances of the differential variances are tested as before by means of the Z test.

Treatment differential v. Parallel differential.

The differential variance for treatment is abnormally small. The comparison with the corresponding parallels differential shows whether treatments have significantly affected the yield curves in time, since the parallels variance is a measure of the errors associated with the differential response. The data give $Z = -1\cdot0894$. The significant value for Z, $P = 0\cdot05$, is $0\cdot1317$, consequently, not only is the treatment differential insignificantly low, but the probability of such a low value being again reached is exceedingly small. Since treatments throughout are merely hypothetical, this result is to be expected. The treatments differential is really only an independent estimate of the parallels differential, but because of its sub-normal value it has not, in further consideration of the latter, been amalgamated with the parallels differential.

Parallels v. Parallels differential.

This comparison furnishes evidence as to the stability of the contributions to parallel variance from occasion to occasion. If the differential variance is small and insignificant, the correlation between occasions will be judged significant for pluckings of the same plots apart from any effect of treatment. In this case $Z = 0\cdot5647$ and the significant value for a 1 in 100 probability that the superiority of parallels is due to chance is $0\cdot5165$. The correlation is thus established beyond doubt and the data are throughout concordant.

Rows v. Rows differential.

A similar process leads to the conclusion that the rank that each row assumes in the various pluckings is significantly constant:

$Z = 1\cdot6214$ as against Z significant less than $0\cdot7086$, where $P = 0\cdot01$.

Columns v. Columns differential.

$Z = 0\cdot6182$: for the different levels of probability the significant figure is less than $0\cdot5073$, $P = 0\cdot05$, and greater than $0\cdot6651$, $P = 0\cdot01$. The probability that the correlation is negligible accordingly lies between

1 in 20 and 1 in 100. In view of this diminished value it is of interest to compare directly the row and columns differentials.

Column differential v. Row differential.

$Z = 0.1426$; Z significant ($P = 0.05$) $= 0.1317$. The column variance is significantly greater than the row variance. The two variances are directly comparable, since rows and columns have the same mean.

Studied in the light of experimental design, the last three comparisons provide an interesting sidelight on the technique of tea experiments. The question of importance is, to what cause can the higher differential variance of columns be attributed? If we consider possible soil effects first, it must be borne in mind that row variance measures variation down the steep slope of the ground, whilst the columns contour is relatively even. This arrangement explains both the general diversity of row behaviour as a whole, with its relatively large primary variance, and the small column variance, if we stipulate a corresponding presence and absence respectively of variable drainage and soil depth, not unlikely characteristics. There seems, however, to be no reason why differential variability due to soil causes should be more marked across the field than down; in fact, it is easier to make out a case for the opposite effect.

One looks, therefore, for some disturbing factor that is present in the columns but not in the rows. Just such a factor is introduced by the system of plucking described in Section (1). All rows are plucked by the same team and any falling away from the standard of plucking affects each row equally. The columns are plucked by different teams; so long as each team maintains from occasion to occasion its own standard of plucking, discrepancies between standards are reflected in and eliminated by the primary column variance. If, however, the standard of a team varies from plucking to plucking, but not to the same degree in each band, the result will show up in the differential variance under discussion.

The possibility of reaching only a variable standard of plucking was referred to in the description of the growth and yield cycle. In view of the actual trend of the data further details are worth considering. Bad plucking consists of either over- or under-plucking, the former being the more prevalent, since a portion of the pluckers' wages is calculated on a poundage basis. Under-plucking gives a low yield on the first occasion, but if normal plucking follows potential yield is not sacrificed. Frequent under-plucking would thus produce that fluctuation in column yield which, in comparison with what was happening on other columns, would produce a differential variance with occasion. Over-plucking involves

the augmentation of the first yield at the expense of the second, since the number of semi-mature shoots left for development is reduced; at the same time a limit is imposed upon further similar treatment. Here again a pendulum action is produced. In this investigation, possibly both causes operated, since plucking was purposely paid at a flat rate. Inasmuch as this encouraged under-plucking it was counterbalanced by periods of greater watchfulness on the part of the field supervisors. It cannot, however, be claimed that these periods were constant for all bands at one time. The result is the inevitable differential plucking from time to time and from column to column which the data seem to support.

The foregoing may be summed up by saying that the yield curves for the 42 occasions behave normally when treatment and rows are considered; that columns, whilst not transgressing the limits of consistent behaviour, show greater variation than the other categories, and that the technique of plucking offers a reasonable explanation of the established variability.

(4) Intermittent plucking.

The consistency in behaviour from plucking to plucking revealed in the previous section suggests the possibility of conducting experiments by means of sample pluckings instead of recording every plucking round. The labour involved in obtaining some 36 yield records a year is considerable. At a central research station complete data are advisable, but at sub-stations intended to collect data at the diverse altitudes and under the equally diverse climatic conditions encountered in tea culture, some curtailment of labour in this direction would make it possible to conduct a larger number of experiments at an increased number of stations. Such experiments would be based on those at the central station for which complete records were available.

The procedure would consist of plucking each round (which would be done in any case by estate labour), but the weighings of the individual pluckings would be restricted to a definite selection of occasions, say once a month. Owing to the variable performance of the plots at different times of the year and at different periods in the pruning cycle, no estimated yields for the whole period would be available, as is possible by the space sampling method on annual crops(15). The comparison of treatments would nevertheless be justifiable if it could be shown that the correlation between performance and error respectively on the two plans was satisfactory. Absolute yields are the least important part of experimental data, since they must vary from soil to soil and climate to

climate. Relative yields from samples over a whole seasonal and pruning cycle would offer a perfectly fair basis for agricultural comparison and advice.

In the following example total yields are compared with the yields of every third occasion, *i.e.* totals of 42 rounds against totals of 14. The time interval is roughly a month between samples. The example chosen is the 6×6 latin square (Table III), which is near optimum plot size, and gives a reasonable number of degrees of freedom for the estimate of error. No new sources of sampling error are introduced in the procedure, since the method is uniform for both total and occasional yields. The occasional yield totals are given in Appendix B; they give the following analysis.

Table VII. *Analysis of occasional pluckings. Mean* $= 1344 \cdot 84$.

Variance due to	Degrees of freedom	Sum of squares	Mean square
Treatment	5	28351	5670
Rows	5	428180	85636
Columns	5	138149	27630
Parallels	20	448264	22413
Total	35	1042944	

From the total and occasional yields the covariance is calculated with the results shown in Table VIII. The appropriate correlations are obtained from the covariances in conjunction with the sums of squares of the two variables.

The correlations between occasional and total pluckings are all high and significant, judged by the standard for a 1 per cent. probability. Of particular interest are those for treatment and parallels which indicate that the comparison of treatment means of occasional pluckings is justifiable and that the elements of error associated with them are comparable also.

A direct test of the errors inherent in the two methods can be made by means of the relative variances, $\dfrac{\text{Variance}}{\text{Mean}^2}$ (15):

Relative variance $\times 100$		$\log_e$
Total yields	1·1544	0·14359
Occasional yields	1·2393	0·21444
	Difference	0·07085
	Z	0·03543

The Z test shows that even for a low probability of 0·05, the two estimates of variance are of the same order, the significant value of Z being 0·3758. The success of the method depends on the changes of the yield curve being slow, so that the intermittent pluckings do not miss

the general characteristics of the whole data. The plucking of every third round for weighing seems, on the evidence of the data, to fulfil this condition, and to give results which allow of valid comparisons without an appreciable loss in accuracy.

Table VIII. *Analysis of covariance.*

Covariance due to	Sums of products	Correlation coefficient	Significant value, $P = 0.01$
Treatment	73493	0·9240	0·9172
Rows	1089977	0·9809	0·9172
Columns	334408	0·9417	0·9172
Parallels	1232721	0·9850	0·5487
Total	2730599		

(5) The utility of previous crop records.

In experiments where, on account of soil or plant heterogeneity, the residual error is high, attempts are frequently made to improve the value of the data by comparing actual yields in the experiment with "calculated" yields derived from previous cropping. The assumption is that though parallel plots are not equally productive in yield, the factors causing the disparity are persistent and approximately constant. In deriving the calculated yields totally inadequate data are sometimes used, as for example in the so-called "percentage increase" method, where the correction employed for all plots is the crude proportionality between experimental and previous yields on a small number of control plots.

Sanders[14] has recently put on a firm basis the method of using previous yields by employing the regression of experimental yields on previous yields for all the plots concerned. Using an equation of the form $y = bx$, where y is yield in the experimental period and x the previous yield, he derives a new variance of y corrected for x and shows that this new variance $Vy.x$ satisfies the equation $Vy.x = Vy - \dfrac{(\text{Covariance } xy)^2}{Vx}$.

The new variance may be used directly to test the significance of treatment as a whole: if differences between treatments are under consideration the comparison must be between mean values of $y - bx$ and not of y. Sanders, using a randomised block experiment, tests all significances with respect to a remainder or parallel variance; consequently, the method involves the calculation of the three statistics Vy, Vx and $\text{Cov } xy$ in the several categories of treatment, position, parallels and total.

In his paper Sanders investigates a uniformity trial on annual crops in which each year's data relate to a different crop. In one case he found

the correlation between experimental and previous yields so small that no reduction of error was obtained. In a second case, the experimental yields, corrected on the basis of the means of three previous croppings, gave a substantial and maximum reduction. The reduction in standard error quoted is from 4·5186 per cent. to 2·5370 per cent. On the basis of these results it may be doubted whether for annual crops the method is really suitable. The successful case quoted has involved the compilation of yield records on all plots for four years. Assuming that errors of the experimental year fairly represent the order of accuracy attainable, two alternatives may be considered:

(a) If the experiment had been done with four times as many replicates in the experimental year, the expectation of error would have been half of 4·5186 or 2·2593 and the significant difference between means would have been 6·47. It will be observed that these figures compare favourably with those obtained by the use of the regression which are 2·5370 for error and 7·26 for significant differences. The extra trouble involved is limited to manuring (in a manurial trial) and the result is obtained in one year instead of four.

(b) If the experiment had been carried out as an experiment with appropriate treatments in each of the four years, a substantial error reduction would have resulted provided that differential seasonal responses were negligible, and if such were not the case there would be the compensating advantage that the results related to average responses of the crop to a variety of seasonal conditions.

In applying the method to permanent crops, it is reasonable to suppose that some of the disadvantages inherent in the use of the method on annual crops will be absent. A higher correlation may be expected from successive yields which are not only of the same crop, but from the same population of individuals within the plots. The improvement in the value of the regression function will be specially beneficial if it reduces error by an amount greater than could have been attained by a replication equivalent to the duration of the uniformity trial. Under these (not unfair) conditions the method would give a more accurate result with a less expenditure of labour.

Sanders' method has accordingly been tested on the limited data here assembled. There is a relatively high residual variance to be reduced (Section (2)), and evidence that the correlation between successive pluckings is high has been given under Section (3). The 42 pluckings have been divided into three groups of 14, and the total yield per group for each plot has been expressed as a percentage of the mean of all plots as set

out in Appendix E; the 4 × 4 latin square is taken as the example. The three periods correspond roughly with three weather periods, the first and third being s.w. monsoon periods and the second a N.E. monsoon with a dry period at the end. By treating the data in this way something similar to seasonal response in annual crops is introduced into the current example.

The third group of yields is taken as the "experimental" yield y for which the analysis of variance is as follows:

<div align="center">Table IX.</div>

Variance due to	Degrees of freedom	Sum of squares	Mean square	S.E.
Treatment	3	158·5	52·83	
Parallels	6	716·5	119·42	10·93
	9	875·0	97·22	
Rows	3	1095·5	365·17	
Columns	3	69·5	23·17	
	15	2040·0		

For the present it is sufficient to consider the parallel variance derived from nine degrees of freedom, that is, neglecting hypothetical treatments; the value is 97·22. From similar analyses of previous yields corresponding values of the variance of x and the covariance xy are found to be as follows:

	Parallel variance x	Parallel covariance xy
2nd group	64·06	72·69
1st group	52·89	48·78
1st and 2nd groups (mean)	48·33	62·72

Substitution of these values of variance for y, x and xy in the Sanders formula gives the following values for $Vy.x$ when the correction for the one degree of freedom absorbed in taking out a linear regression is made:

	$Vy.x$	Relative precision
Corrected for 2nd group	16·57	5·87
„ 1st group	58·76	1·65
„ 1st and 2nd groups	17·81	5·46

The precision scale is relative to the variance of y, the experimental yield, which is taken as unity.

It is evident that a very large reduction in error is effected by the application of this method. The best results are obtained by correcting for the period immediately preceding that used as the experiment. The correction for the first group gives disappointing results considering that .

the conditions of the environment for x (1) and y (3) are very similar. It is possible that when yields are grouped together in this fashion, slow changes taking place toward the end of the pruning cycle are accentuated. Nevertheless, in all three cases the error reduction is substantial. An example of an experiment involving the use of treatments, parallel to that of Sanders, may be derived from the consideration of the third period (y) corrected for the second period (x).

The regression coefficient b in the equation $y = bx$ ordinarily defined as $b = \dfrac{r \cdot \sigma_y}{\sigma_x}$ is equal to $\dfrac{\text{Cov } xy}{Vx}$ in terms of statistics already calculated, and gives a value of $1 \cdot 1615$ for a 4×4 latin square with treatments. Applying this to treatment means, we have:

Treat-ment	Mean yield y	Mean yield x	bx	$y - bx$
A	99·75	101·75	118·18	− 18·43
B	101·00	100·50	116·73	− 15·73
C	95·25	95·75	111·21	− 15·96
D	104·00	102·00	118·47	− 14·47

The treatments are again hypothetical and it is evident from the values of $y - bx$ that there has been a levelling up. The analysis of variance of $y - bx$ (*i.e.* when the regression is introduced) is as follows:

Table X.

Variance due to	Degrees of freedom	Sum of squares	Mean square	S.E.
Treatment	3	32·93	10·98	
Parallels	5	87·86	17·57	4·19
Rows	3	156·23	52·08	
Columns	3	77·03	25·68	
Total	14	354·05		

A comparison can now be made between the errors derived from the crude yields and those from the corrected yields (Tables IX and X). Translated into terms of significant differences between means of treatments by the use of the statistic t, we have:

	Without regression	With regression
s.e. of plot	10·93	4·19
s.e. difference of means of 4	7·727	2·961
Significant differences $P = 0.05$	18·91	7·61
$P = 0.01$	28·64	11·94

In terms of the precision index, the experiment with the regression is 6·81 times as accurate as that without; since the replication is fourfold, the corrected figures give an accuracy which could only be expected

from the crude data with a replication of twenty-seven and with the use of the regression this involves only twice the labour.

SUMMARY.

1. A uniformity trial of 144 plots of mature tea plucked on 42 occasions is examined with a view to determining an adequate technique for field experiments.

2. Yields are expressed as dry matter; the necessity for, and accuracy of, this procedure is examined.

3. Using the latin square and statistical analysis of variance, a maximum diminution of error up to the plot sizes of $\frac{1}{18}$ acre is found; the elimination of positional variance is shown to be inferior to that generally obtained on annual crops in temperate climates, but this inferiority corresponds with similar experience on other tropical crops.

4. An examination of the errors of yield curves over the whole of the separate occasions reveals a significant consistency between behaviour on successive occasions, differential variances with respect to occasions being small. That for columns is significantly greater than that for rows, and on consideration of the arrangement of the experiment this greater value is attributed to variation in the standard of plucking attained on the various occasions.

5. The use of intermittent yields (every third plucking round) as a labour-saving device, gives valid comparisons between treatments without an appreciable increase in error.

6. The application of Sanders's method for correcting experimental yields on the basis of previous cropping gives satisfactory results of greater promise than in the case of annual crops.

The literature devoted to field experiments contains few examples of uniformity trial data from which crop records are available over an extended number of harvests. When various experimental designs and the statistical methods relating to them are under investigation, the need of trial data is frequently felt. In the hope that the records accumulated in this investigation may be of use in this connection, the complete primary data have been deposited in the library of the Rothamsted Experimental Station, and will be published later as *Bulletin* No. 6 of the Tea Research Institute of Ceylon. Copies may be had on application to the Institute.

The writer is indebted to the Manager of the Ceylon Tea Plantations Co., Ltd., and to the Superintendents of The Scrubs Estate, Nuwara

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APPENDIX A.

12 × 12 latin square. Total yields.

1	2	3	4	5	6	7	8	9	10	11	12	Total
3 827·0	*4* 751·4	*7* 904·4	*5* 852·5	*2* 1002·4	*8* 1048·6	*6* 960·6	*1* 868·0	*10* 686·0	*12* 805·8	*11* 889·2	*9* 811·0	10406·9
10 877·3	*3* 797·9	*1* 949·5	*12* 795·2	*11* 891·3	*2* 971·6	*9* 1003·8	*6* 759·3	*4* 887·7	*5* 916·8	*7* 739·6	*8* 760·8	10350·8
7 977·1	*9* 857·5	*12* 993·5	*2* 974·6	*6* 1005·7	*10* 1010·8	*8* 972·8	*3* 880·6	*5* 722·4	*11* 722·1	*1* 778·6	*4* 962·2	10857·9
5 944·8	*1* 863·8	*11* 1050·8	*8* 945·1	*10* 1005·3	*9* 1278·0	*3* 1240·7	*12* 1147·7	*6* 1056·0	*2* 1262·3	*4* 1363·2	*7* 1109·3	13267·0
8 877·5	*12* 1041·9	*6* 966·8	*9* 1257·4	*5* 1151·4	*3* 1101·8	*7* 1021·2	*10* 952·2	*11* 827·7	*4* 813·0	*2* 900·1	*1* 1022·8	11933·8
4 841·0	*2* 840·5	*9* 837·3	*1* 892·8	*3* 1014·4	*7* 829·9	*11* 1064·7	*8* 1095·5	*12* 1165·6	*10* 1227·9	*6* 1504·6	*5* 926·1	12240·3
6 1236·5	*10* 1211·2	*2* 1111·1	*7* 1196·6	*4* 1205·0	*1* 1216·4	*5* 1361·9	*9* 1371·4	*8* 1428·3	*3* 1428·2	*12* 916·4	*11* 703·4	14386·4
1 912·9	*5* 967·6	*8* 884·8	*4* 1122·5	*12* 1076·2	*11* 993·3	*10* 1115·3	*7* 981·1	*2* 953·5	*9* 926·8	*3* 740·6	*6* 1038·0	11712·8
9 1140·5	*8* 1092·9	*4* 968·3	*3* 975·0	*1* 824·3	*5* 716·6	*12* 840·4	*11* 883·5	*7* 968·5	*6* 692·5	*10* 861·5	*2* 962·7	10926·7
11 860·0	*7* 1000·6	*3* 966·8	*6* 958·6	*9* 1034·6	*12* 1092·5	*2* 1211·5	*4* 1133·4	*1* 712·4	*8* 603·9	*5* 676·9	*10* 1029·0	11280·2
2 986·4	*6* 823·4	*10* 824·1	*11* 794·3	*8* 990·9	*4* 1040·6	*1* 943·9	*5* 738·9	*3* 716·9	*7* 993·3	*9* 1163·1	*12* 953·4	10969·2
12 841·9	*11* 763·2	*5* 846·1	*10* 995·5	*7* 942·1	*6* 1215·7	*4* 1169·2	*2* 747·1	*9* 681·7	*1* 964·8	*8* 1093·9	*3* 1457·4	11718·6
11322·9	11011·9	11303·5	11760·1	12143·6	12515·8	12906·2	11558·7	10806·7	11357·4	11627·7	11736·1	140050·6

APPENDIX B.

6×6 *latin square. Total yields in ordinary type.*
Intermittent yields in heavy type.

4	*1*	*5*	*6*	*3*	*2*
3253·6	3501·6	3913·9	3591·7	3296·3	3200·6
1105·5	**1199·1**	**1296·4**	**1286·9**	**1047·1**	**1082·2**
6	*3*	*2*	*1*	*4*	*5*
3643·2	3964·0	4299·8	4241·8	3762·8	4213·3
1241·3	**1337·9**	**1408·2**	**1502·1**	**1241·0**	**1444·7**
2	*6*	*1*	*4*	*5*	*3*
3600·9	3954·3	4097·5	4133·6	4034·2	4353·6
1268·6	**1386·1**	**1425·9**	**1423·5**	**1353·0**	**1473·8**
3	*4*	*6*	*5*	*2*	*1*
4328·2	4315·0	4490·9	4829·9	4736·8	3398 4
1579·0	**1489·1**	**1537·7**	**1733·1**	**1662·1**	**1200·4**
1	*5*	*3*	*2*	*6*	*4*
4094·0	3868·7	3668·0	4068·8	2977·3	3530·1
1463·0	**1375·4**	**1310·4**	**1425·1**	**1050·2**	**1226·5**
5	*2*	*4*	*3*	*1*	*6*
3414·9	3460·0	4189·3	3599·1	3356·7	4667·8
1201·6	**1180·9**	**1427·1**	**1260·8**	**1128·3**	**1640·3**

APPENDIX C.

4×4 *latin square. Total yields.*

3	*1*	*2*	*4*
7935·6	8552·7	7741·2	7386·1
1	*3*	*4*	*2*
8264·4	9476·1	9571·3	10129·3
4	*2*	*1*	*3*
9525·8	9325·9	9904·1	8270·1
2	*4*	*3*	*1*
7912·5	9064·8	8055·0	8935·7

APPENDIX D.

3×3 *latin square. Total yields.*

3	*1*	*2*
14362·4	16047·2	14473·0
1	*2*	*3*
16198·4	17551·9	16523·0
2	*3*	*1*
14837·6	15525·2	14531·9

APPENDIX E.

4×4 *latin square. Group yields as percentage of mean for each group.*

1st line	Group 1.
2nd line	Group 2.
3rd line	Groups 1 and 2.
4th line	Group 3.

3	*1*	*2*	*4*
95	95	88	83
88	102	91	88
91	100	90	86
90	**93**	**85**	**81**
1	*3*	*4*	*2*
96	108	105	106
94	110	109	118
95	109	107	113
93	**106**	**114**	**121**
4	*2*	*1*	*3*
103	109	113	96
109	105	115	94
107	107	114	95
114	**106**	**111**	**93**
2	*4*	*3*	*1*
93	103	95	112
88	102	91	96
90	102	92	102
92	**107**	**92**	**102**

THE INFLUENCE OF MANURIAL TREATMENT ON THE BAKING QUALITY OF ENGLISH WHEAT.

I. A QUALITY STUDY OF THE ROTHAMSTED BROADBALK WHEATS.

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(With Plates III and IV.)

In any enquiry as to the possibility of influencing the quality of English wheat by means of manurial treatment the Broadbalk Field of the Rothamsted Experimental Station offers an unique opportunity in certain directions. As is well known, the respective plots of the Broadbalk Field have received constant manurial treatment under the continuous growth of wheat for a lengthy period of approximately ninety years. The dosages, moreover, have been very heavy. The soil of each plot, and the wheat grown upon it, might therefore be expected to show to an unusually marked degree the effect of the particular fertiliser to which it has been subjected.

Precise records of the yields of wheat and straw from the various Broadbalk plots throughout the years have been kept at Rothamsted, but the samples have not been tested in the bakehouse except in 1903 and 1904 when trials were made on the crops of those years by Dr A. E. Humphries[8] and the Home-grown Wheat Committee of the National Association of British and Irish Millers. We have received each season since 1926 bushel samples of certain of the Broadbalk wheats. These samples have been milled on our experimental mill, and the straight-run, or 100 per cent., flours tested in the bakehouse according to our usual procedure.

It is unnecessary here to describe in detail the Broadbalk wheat-growing. It will suffice to recall the manurial treatments and give the yield of wheat per acre and the nitrogen contents of the grain, crop by crop, for the various plots we have tested. This is done in Table I. It will be noticed that one or two plots have been dealt with in certain seasons and not in others. The plots of particular interest from the point of view of baking quality are those featuring nitrogenous manuring in different

amounts and forms. These include Plots 2, 3, 6, 7, 8, 9 and 16, and it is obvious from the information as to manurial treatment in Table I that the following comparisons should be of particular interest:

> (a) 6, 7 and 8,
> (b) 9 and 16,
> (c) 6 and 9,
> (d) 7 and 16.

Table I shows that the nitrogen contents are increased consistently only by the heaviest nitrogenous treatments. The lowest nitrogenous treatments (cf. Plots 6 and 9 which show closely similar nitrogen contents) do not significantly raise the nitrogen content of the wheat as compared with the unmanured, and they actually lower the nitrogen content in comparison with that of the plots dressed with complete minerals only. In this connection it must be remembered that the yield per acre of grain is increased by the lowest nitrogenous treatments. It will be noticed that the farmyard manure in the dosage applied has as great an effect upon nitrogen content as the heavier dosages of the artificial nitrogenous fertilisers.

The type of wheat grown throughout these trials was Red Standard in 1926, 1927 and 1928 and Squarehead's Master in 1929. In 1926 a change was made in the hitherto unbroken routine of cultivation. Up to that year the wheat had been grown continuously and the unrested land had become exceedingly dirty in the agricultural sense. Conditions were so bad that in 1926 and 1927 one half of the field was fallowed and the 1928 and 1929 crops were grown on these fallowed portions. As a result yields were increased, and the appearance and condition of the grain were improved almost out of recognition. The change in procedure can be discerned in Table I: the nitrogen contents of the 1928 and 1929 crops tend to be consistently lower than those of the preceding seasons; the yields on the other hand are strikingly higher.

In Table II are given nitrogen contents and "maltose figures" for the flours of the four seasons' crops. The maltose figure is the percentage of reducing sugar found after incubating 20 gm. of flour with 160 c.c. of water for 1 hour at 27° C. The figure includes the reducing sugar originally present and that formed during incubation. The figure is a good indication of soundness of grain, since even a slight degree of sprouting would cause a marked increase in diastatic activity and hence in maltose figure. In most normally sound wheats the maltose figure as conventionally determined is usually below 2 per cent. It is evident from Table II that

Table I. *Details of manurial treatment, yield of grain per acre and nitrogen contents of the Broadbalk wheats for the four seasons 1926, 1927, 1928 and 1929.*

Plot	Manurial treatment	1926 crop		1927 crop		1928 crop		1929 crop	
		Yield of grain* (bushels per acre)	Nitrogen content† (%)	Yield* (bushels)	Nitrogen content (%)	Yield* (bushels)	Nitrogen content (%)	Yield* (bushels)	Nitrogen content (%)
2 A	Farmyard manure (14 tons per acre)	6·8	—	19·5	2·08	41·1	1·84	23·3	—
2 B	Farmyard manure (14 tons)	6·5	1·67	24·2	1·97	48·4	1·96	30·0	1·75
3	Unmanured since 1839	0·9	1·29	6·9	1·74	27·9	1·56	9·1	1·51
5	Complete minerals‡: no N	2·2	1·28	6·5	1·70	35·2	1·70	9·1	—
6	Ditto: +206 lb. Am$_2$SO$_4$§	5·9	1·32‖ / 1·34‖	12·5	1·63	47·3	1·62	17·7	1·54
7	Ditto: +412 lb. Am$_2$SO$_4$	5·7	—	21·5	1·93	67·4	1·70	20·9	1·68
8	Ditto: +618 lb. Am$_2$SO$_4$	7·5	1·69	25·9	2·08	57·2	1·84	15·9	1·80
9	Ditto: +275 lb. NaNO$_3$§	5·8	1·48	16·6	1·70	56·1	1·59	21·6	1·58
10	412 lb. Am$_2$SO$_4$: no minerals	4·4	1·46	12·0	1·80	47·0	1·69	24·7	—
11	Ditto: + 3½ cwt. superphosphate	4·2	1·40¶ / 1·38¶	8·9	1·97	56·9	1·58	19·0	—
12	Ditto: +3½ cwt. superphosphate+366 lb. Na$_2$SO$_4$	7·1	1·43	13·5	2·07	57·3	1·55	22·9	—
13	Ditto: +3½ cwt. superphosphate+200 lb. K$_2$SO$_4$	9·3	1·61	17·4	1·86	55·2	1·64	25·6	—
14	Ditto: +3½ cwt. superphosphate+280 lb. MgSO$_4$	8·6	1·52	16·3	1·94	58·6	1·60	23·4	—
16	Complete minerals+550 lb. NaNO$_3$§	7·5	1·63	18·1	2·06	56·1	1·80	26·3	1·70

* Taken from *Rothamsted Annual Reports,* 1926–9.
† In 1926 of flour only; in 1927–9 of wheat.
‡ Complete mineral manure =3½ cwt. superphosphate, 200 lb. sulphate of potash, 100 lb. sulphate of soda, 100 lb. sulphate of magnesia.
§ Sulphate of ammonia is applied as to one-third in autumn and two-thirds in spring. Nitrate of soda is all given in spring, there being two applications at an interval of a month on Plot 16.
‖ Independent millings; flour yields, 63·4 and 65·7 per cent. respectively.
¶ Independent millings; flour yields, 60·5 and 66·4 per cent. respectively.

in none of the crops was unsoundness of grain a factor in the poor quality observed in many of the flours.

Before proceeding to give a brief account of the broad features and relative behaviour in the bakehouse of each year's crops, it is necessary to point out the importance of adequate gas production in doughs during fermentation, and especially during the final, critical, proving period, *i.e.* the period when the loaves have been moulded and are waiting to go into the oven. The importance of this gas production as a factor in producing large loaves was first pointed out by T. B. Wood(9) in 1907. The later work of A. E. Humphries(5, 6, 7) and his collaborators showed that the quantity of gas given off in final proof does not determine the size of the loaf; it is rather a necessary condition for the production of a large loaf. It is obvious that a large loaf cannot result if gas production during proof is inadequate. Sufficient gas must be produced; then, if the other contributing factors are present (*e.g.* satisfactory gluten characters), a loaf of large volume may be made.

Table II. *Nitrogen contents and maltose figures of the Broadbalk flours*, 1926, 1927, 1928 *and* 1929 *crops.*

| | 1926 crop | 1927 crop | | 1928 crop | | 1929 crop | |
| | N | N | Maltose | N | Maltose | N | Maltose |
Plot	%	%	%	%	%	%	%
2 A	—	1·67	1·56	1·63	1·4	—	—
2 B	1·67	1·74	1·36	1·75	1·4	1·55	1·75
3	1·29	1·42	1·10	1·42	1·4	1·31	1·51
5	1·28	1·44	0·89	1·50	1·3	—	—
6	1·33	1·41	1·15	1·43	1·3	1·34	1·54
7	—	1·69	1·55	1·51	1·6	1·44	1·68
8	1·69	1·76	1·37	1·65	1·5	1·59	1·80
9	1·48	1·45	1·10	1·44	1·2	1·34	1·58
10	1·46	1·65	0·85	1·46	1·1	—	—
11	1·39	1·70	1·37	1·33	1·3	—	—
12	1·43	1·59	1·12	1·38	1·2	—	—
13	1·61	1·62	1·38	1·44	1·0	—	—
14	1·52	1·68	1·33	1·37	1·4	—	—
16	1·63	1·71	1·41	1·62	1·3	1·37	1·70

The power to gas freely and for a long period is a varietal characteristic in the sense that when grown under comparable conditions some varieties of wheat are always better gassers than others(5c). A given variety, however, may vary widely in gassing power from season to season, or according to environment during growth.

The importance of the point lies in the fact that unless adequate gas production can be ensured faulty conclusions may be drawn from baking tests: thus a flour of good gluten quality may behave in the bakehouse

as badly as one of poor quality if gas production is insufficient. Fortunately, Humphries has shown that gas production can usually be increased sufficiently by the addition of small quantities (*e.g.* 0·1 per cent.) of highly diastatic malt extract with or without the further addition of acid ammonium phosphate (0·05 per cent.). In the baking tests to be described the usual baking process adopted was a fermentation of 3¼ hours (including final proof) with 2 per cent. yeast and a dough temperature of 80° F. In some cases 8 hours' fermentation with 0·5 per cent. yeast was employed. With these processes it is important that sufficient gas be produced to inflate properly the dough (besides overcoming the considerable leakage that occurs from all doughs) in the last ¾ hour (*i.e.* between 2½ and 3¼ hours) when 2 per cent. yeast is used, and in the last hour (*i.e.* the eighth hour) when only 0·5 per cent. of yeast is employed. Gas production figures for four seasons' Broadbalk wheats were determined, but need not be given here. Gas production was maintained at a high and satisfactory level for 4 to 5 hours with 2 per cent. yeast, and for 8 hours with 0·5 per cent. yeast in the wheats of 1927, 1928 and 1929. The 1926 crop was definitely less satisfactory in this respect. With 2 per cent. yeast gassing was good for the first 3 hours, but a sharp fall occurred in the fourth hour. Gas production, therefore, in the bakehouse was probably satisfactory during the whole 3¼ hours, and this is supported by the baking results given in Table III; although the two best loaves came from two of the three best gassers, the worst gassers did not produce the worst loaves. Broadly speaking, the baking results for the 1926 crop are probably comparable *inter se*. With the later crops malt extract and ammonium phosphate were used when necessary; the results for the three years 1927, 1928 and 1929 are therefore strictly comparable.

<div align="center">THE 1926 CROP.</div>

The wheats were of fairly high moisture content when received, and the mill feeds had moisture contents ranging from 17·1 to 18·4 per cent. With one or two exceptions the extractions or flour yields were very similar but low. This is because the work on this crop was carried out before our milling technique was modified in the direction of getting a better "finish" on the offals. In any case moderate differences in extraction in no way invalidate comparison between the plots. In one case (Plot 11) two samples of the wheat were milled independently with a deliberate difference in extraction of 6 per cent. (11 (i) 60·5 per cent.; 11 (ii) 66·4 per cent.), but the baking results were very similar with both
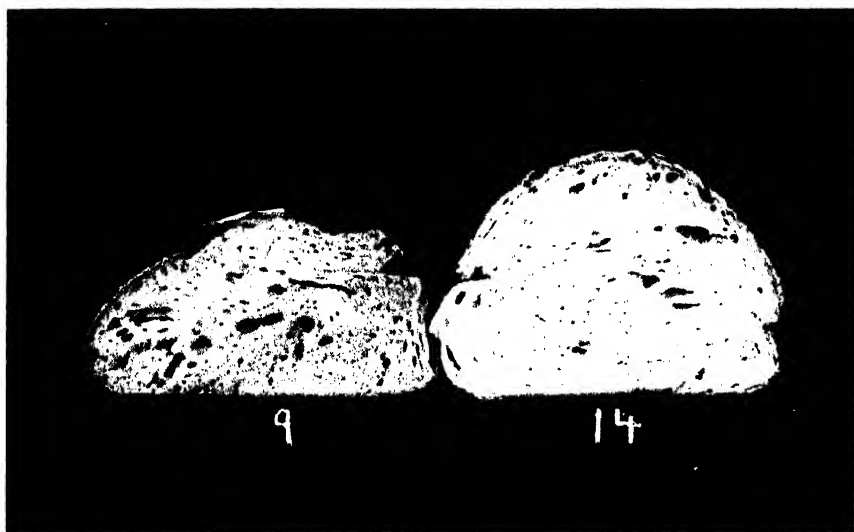
A. Worst and best loaves from 1926 crop of Broadbalk wheats.
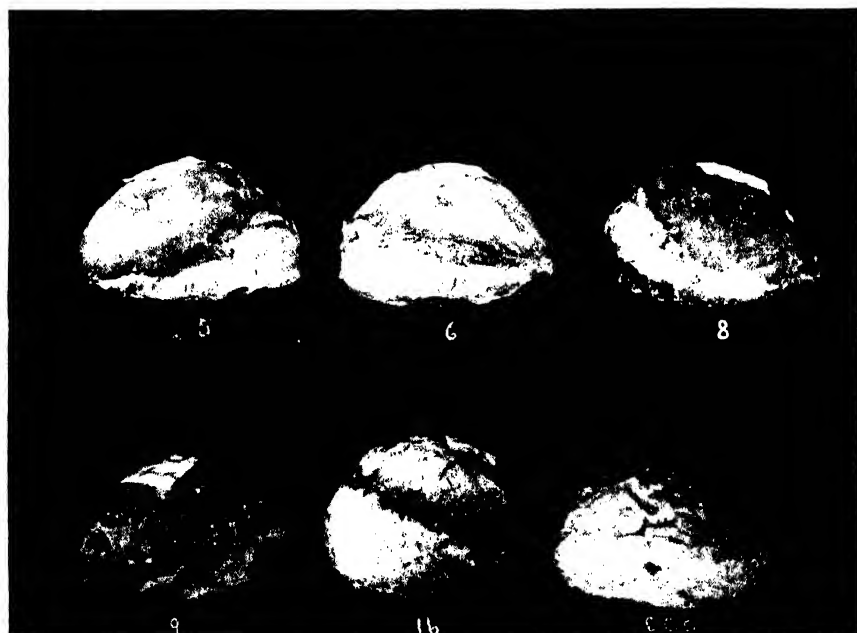
B. Typical loaves from 1926 crop of Broadbalk wheats. Compare the torn inferior crusts with the unbroken and nicely checked crust of CCC, which is a good London flour made from a mixture of wheats.

samples[1]. It may be said, in general, that in a given year any differences in baking quality between flours milled from the respective plots which may be due to differences in the flour yields are so small as to be negligible in comparison with the relatively large differences due to variations in the quality of the wheats themselves.

The wheats of the 1926 crop were milled on various occasions between January 25 and March 28, 1927. In order to minimise any differences due to varying "age" of the samples the flours were not baked until May 31, 1927, when tinned loaves were made. With the exception of Plot 11 the flour extractions obtained ranged between 63 and 65·7 per cent. The flour from Plot 9 was very dirty: it was impossible to clean the wheat entirely satisfactorily on our small dry-cleaning plant before milling. It is not quite certain to what extent this circumstance influenced the baking results obtained on the 1926 crop from Plot 9.

The bread as a whole must be described as definitely inferior to that from average all-English non-Yeoman flour—unappetising in appearance, broken and very pale in crust with lifeless, coarse crumb of a greyish colour (cf. Plates III, A and B). Nevertheless, it was better than that obtained from Broadbalk crops of some later seasons.

Nos. 11 (i), 11 (ii) and 13 were outstandingly the best loaves. Their volume and oven spring were, indeed, good for all-English bread and their crumb was of fair all-round quality—its weakest features being spring and colour. The other extreme is represented by 2, 3, 5, 6 and 20, which were poor loaves with crumb which was "corky" and of poor spring. The general order of merit of the loaves is shown in Table III.

A second series of baking tests was carried out on August 3, 1927, when cob loaves were baked.

Each sample took liquor at the rate of 15 gallons per sack. The doughs as a whole were of the claylike, "lifeless" type; 9 was worst: it was very tender and sticky, "short" and claylike. The others showed a slight graduation in the order given in Table III, 12, 13 and 20 being best. These were still poor doughs, being short and claylike, but they were much tougher and more elastic than 9.

On the tray during the final proof for the oven considerable differences in stability were observed; 14 and 16 were bold, having very good stability; 11, 12 and 13 had good stability; whilst 9, 3 and 10 were poor. The others had very fair stability.

Of the cob loaves baked on August 3, 1927, 9 had deteriorated badly.

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[1] This difference in extraction, moreover, was effected partly by more thorough cleaning of the bran and not only by heavier treatment of reduction stocks.

Table III. *Showing order of merit of the Broadbalk wheats in the various baking tests on each crop with respect to dough and loaf qualities.*

Crop	Date when baked		General character	Order in decreasing merit of the various plots
1926	31. v. 27	Loaves	—	11 : 13 : 8 : 16 : 12 : 10, 14, 9 : 2 : 3, 5, 6, 20
	4. viii. 27	Doughs	Claylike and short	12, 13, 20 : 14, 16 : 6, 8 : 10, 11 : 3, 5 : 2 : 9
		Loaves		14 : 11	5 : 12 : 16	8 : 3 : 10 : 13 : 6, 20 : 2	9
1927	1. viii. 28* ...	Doughs	Clay-like	2 : 6, 7 : 8	3, 5	9, 10, 11, 12, 13, 14, 16
		Loaves	Superior to 1926	2 : 6, 7 : 3 : 8 : 5, 10, 11, 14 : 12, 13, 16
	16. viii. 28† ...	Doughs	Claylike	2 : 6, 7 : 8	3, 5	9, 10, 11, 12, 13, 14, 16
		Loaves		2, 6 : 7 : 3, 5, 8 : 12	9, 10, 14 : 11, 13, 16
	April, 1929‡ ...	Doughs	Tough and short	7, 8 : 6, 9, 16 : 2, 5 : 3 : 11, 14 : 12, 13 : 10
		Loaves		7, 8, 2, 11 : 6, 12	16, 14 : 9, 13, 5 : 3, 10
1928	April and July, 1929 ...	Doughs	Of different character from previous crops—tender, elastic, poor stability	6 : 3, 2, 5	16 : 8 : 13, 14 : 10, 12 : 11	7, 9
		Loaves	Very poor	
	Blended with No. 1 N. Manitoba flour:			
	January, 1930	Doughs		6 : 5 : 3, 8, 2 : 16, 10, 11, 12, 13, 14	7, 9
		Loaves	All alike except 7 and 9 which were poor	
	July, 1930	Doughs		6, 8 : 10 : 2 : 3 : 11 : 5, 12, 13, 16	9 : 7
		Loaves	As in January	
1929	(Baked alone) 2. vii. 30 ...	Doughs		3, 6, 8 : 2, 13, 16, 5, 11, 12 : 10	9 : 7
		Loaves	Fairly good, tender, elastic	16 : 9 : 2, 3, 7, 8 : 6
			Superior to 1928	16 : 9 : 7 : 6, 3	8 : 2
	Blended with No. 1 N. Manitoba flour:			
	3. vii. 30	Doughs		8§ : 2§, 7, 9, 16 : 3§, 6
		Loaves		16, 9 : 7, 6 : 3	2 : 8

* 0·5 per cent. yeast and 8 hours' fermentation.
‡ 2 per cent. yeast and 3¼ hours' fermentation.
† 1·25 per cent. yeast and 3 hours' fermentation.
§ Poorest stability.

N.B. A vertical line in the table between plots implies a marked difference, a colon a less marked difference, whilst a comma implies no difference in quality between the plots so separated.
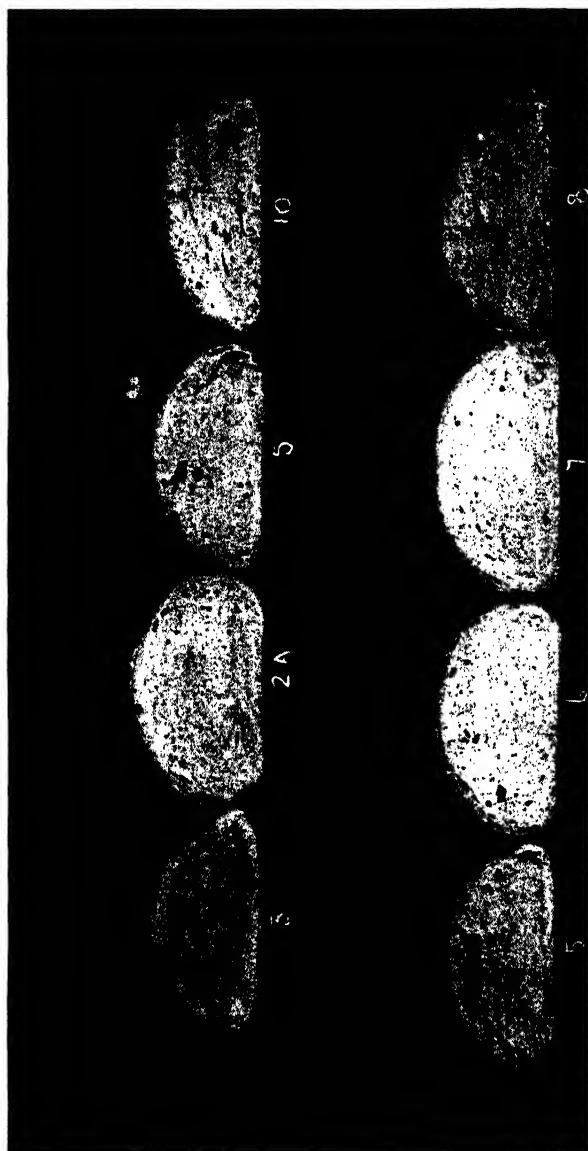
Cob loaves made from Broadbalk flours, 1927 crop. Plots 11, 12, 13, 14 and 16 (not shown in this plate) were alike but inferior to 10.

The general order of merit of the loaves is assessed in Table III, from which it will be seen that whilst certain samples have markedly deteriorated, e.g. 9 and 13, others have improved, notably 14 and 5—at least relatively. The outstanding instance of this latter tendency is 14, which is now the best all-round loaf.

THE 1927 CROP.

The arrival of the 1927 crop, in February, 1928, followed a bad harvest, and the wheats as received had moisture contents ranging from 18 to 21 per cent. This made milling difficult, and accordingly in many cases the samples were partly air-dried at room temperature prior to milling. As good a finish on the offals as possible was obtained, but extractions varied between 65 and 72 per cent. The milling was carried out on various occasions between the middle of February and the end of July, 1928.

The samples were baked on August 1, 1928, using $\frac{1}{2}$ per cent. yeast and 8 hours' fermentation, and on August 16 using $1\frac{1}{4}$ per cent. yeast and 3 hours' fermentation. In both cases cob doughs were made and each was given liquor at the rate of 14 gallons per sack.

Throughout both baking trials corresponding doughs behaved alike. There was considerable variation in quality, but all the doughs were of the lifeless claylike type. Nos. 2, 6, 7 and 8 were easily best and handled satisfactorily. They were tough but "short" and had good stability. The remaining doughs were much poorer and handled very lifelessly. They are grouped according to general quality in Table III.

The bread varied widely in quality. The best loaves were excellent for all-English. Plate IV shows a group which includes the best loaves baked on August 1. Plot 2 gave the best all-round loaf. For all-English bread it had excellent volume and oven spring and good all-round crumb quality. Its crumb, in particular, lacked that "corkiness" characteristic of much all-English bread. It had good spring and a soft smooth texture.

The worst loaves were 12, 13 and 16 (not shown in photograph), which were much alike and rather poorer than 10, 11 and 14, which were also much alike and rather poorer than 5. Nos. 12, 13 and 16 were flat unappetising loaves of very poor, close-grained, cheesy crumb of bad colour.

With one or two exceptions the loaves baked on the shorter system (3 hours with $1\frac{1}{4}$ per cent. yeast) tallied with those on the longer system. The general order of merit is shown in Table III for both baking tests. It will be noticed that 5 and 12 responded better to the short system than to the long.

The 1927 crop was baked again in April, 1929. Cob loaves again were made, the absorption being still 14 gallons. The doughs were now very tough and "short" compared with those of the previous year's bake. Plots 7 and 8 were now best in point of all-round dough quality. They were of good body and tough, but very "short" and claylike. Plots 9 and 16 had improved relatively and were now only inferior to 7 and 8. The doughs were all much of the same type, and there were none which were outstandingly inferior to others.

Many of the samples still made satisfactory bread. Plots 2, 7, 8 and 11 had good oven spring and volume, and crumb which was very close in grain but of satisfactory spring, pile and texture. Plots 6 and 12 were only slightly inferior to these, but the others were a considerable way behind, especially in crumb quality.

The order of quality both of doughs and of loaves is given in Table III.

It will be seen that 11 had markedly improved, whilst 3 had markedly deteriorated.

The 1928 crop.

The 1928 crop was the first outcome of the change in procedure of cultivation described in the introduction, and the improved conditions were at once obvious in the appearance of the wheats. The samples were very much cleaner, and the grain was greatly improved in appearance and milling character.

When received in December, 1928, the samples had moisture contents ranging from 17 to 21 per cent. To overcome as much as possible the difficulty of varying age in the flours owing to the considerable time over which the milling of the various samples had to be spread, the samples were milled and baked in two groups. Comparison was effected through the inclusion of Plot 3 in both groups.

Plots 3, 6, 7, 8, 9 and 16 were milled during the first fortnight in March, 1929, and a baking test carried out in April. Plots 2 A, 2 B and 3 (a second sample), 5, 10, 11, 12, 13 and 14 were milled between the middle of April and the end of May, and baked in July, 1929.

When milled the samples of wheat, which had been stored in bags in a cool dry place, had dried appreciably and on the whole they milled very well, though the moisture contents of the mill feeds ranged between 13 and 18 per cent. The extractions obtained ranged between 70 and 74 per cent.

The change for the better in condition of the wheats was reflected in

the better appearance of the flours and, in turn, in a vastly improved crumb colour in the bread. On the whole, the crumbs had a pleasing creamy white colour—in marked contrast to the lifeless greyish crumb of previous years. These features were accompanied by a radical change in type of the doughs as a whole and, unexpectedly, by a marked change for the worse in general bread quality. Instead of being claylike and lifeless but stable, the doughs were for the most part tender and extensible with poor stability. As with the 1927 crop, absorption was 14 gallons per sack for cob doughs. Plot 6 gave the best dough. It had good body, and was fairly tough and extensible with fairly good spring. Plots 3, 2 and 5 were also satisfactory, but the remaining doughs tended to be excessively tender. Plots 7 and 9 were outstandingly the worst; they were scarcely better than batters, were almost impossible to handle and had to be scaled into tins—it was impossible to bake cob loaves from them. They were obviously unable to carry as much water as the other samples.

All the loaves were more or less flat, lacking boldness—a feature which reflected the inferior stability of the doughs. Altogether the bread was so poor that it was difficult to make satisfactory comparison. However, a guide to the relative value of the plots, both in doughs and bread, is given uniformly with those of other years in Table III.

Plot 6 gave probably the best loaf. It had only fair oven spring and volume, with crumb which was open but even in grain, of only fair spring and rather coarse texture with a creamy white colour. The loaves from Plots 2 A and 2 B were very much alike, and of inferior crumb spring to 6; 7 and 9 were distinctly the worst: these two flours had probably the poorest baking quality within our experience.

An alternative and valuable method of comparison is available even in the case of very poor samples such as these. It consists in blending each sample to the same extent with "strong" flour, such as that from No. 1 Manitoba wheat.

In the present instance 50–50 blends of the flours from the various plots, respectively, with a sample of No. 1 Manitoba straight-run flour were baked on January 16, 1930. Cob loaves were baked, using 2 per cent. yeast and a fermentation time of 4 hours, and 0·1 per cent. malt extract and 0·05 per cent. ammonium phosphate was incorporated into each dough in order to avoid any complications which might possibly arise through inadequate gas production.

Each dough took liquor at the rate of 16 gallons per sack. With the exception of 7 and 9 there were no marked differences between the

handling properties of the blends, all of which had good body and were fairly tough and elastic, and handled tightly with good spring. Plots 7 and 9, however, tended to become unduly tender as fermentation proceeded. They moulded distinctly more tenderly than the others (7 being the poorer of the two) and, alone of the blends, showed inferior stability in the final proof.

Plots 6 and 8 gave the best loaves of all the blends. They had very good oven spring and volume, slightly torn but otherwise good crusts and good outside appearance, very close and even grain of crumb, which was of good spring, pile and texture, with a good pale grey-white colour. Plots 7 and 9 were easily the poorest loaves (7 being poorest of all); 7 had very poor oven spring: it was a flat loaf, with a reddish, torn crust, and crumb (of the "English" type) of a fine honeycomb grain with poor spring and a rather poor though creamy colour.

The plots are arranged in order, according to general quality shown in this baking test, in Table III.

These flours were again blended and baked in exactly the same way on July 4, 1930. Their behaviour in the dough was similar to that during the first series of blending tests. The loaves also on the whole were very similar to those of the earlier tests. Again 7 and 9 were markedly the poorest (7 being the poorer). Plot 3 was now, however, quite as good as 6 and 8. These three loaves were the best and were like the best loaves of the January bake. Differences between the remaining loaves were not marked. The general order is shown in Table III.

THE 1929 CROP.

The arrival of the 1929 crop in February, 1930, followed a good harvest, and the wheats were in excellent condition. As received, they had moisture contents between 15 and 16 per cent. They milled well, extractions varying between 70 and 72·7 per cent.

The range of plots dealt with was restricted this year to the following seven: 2, 3, 6, 7, 8, 9 and 16, which were of greatest interest from the point of view of nitrogenous manuring. The samples were milled during March and April, 1930, and baked on July 2, 1930.

Each sample was given liquor at the rate of 16 gallons per sack, which, allowing for the fact that tinned doughs were made, indicated a distinctly higher absorption than that of previous years. $3\frac{1}{4}$ hours' fermentation (with 2 per cent. yeast and $1\frac{1}{4}$ per cent. salt) was used and 0·1 per cent. malt extract and 0·05 per cent. ammonium phosphate was added to · each dough.

The doughs as a whole were fairly good in handling properties, and were of the same type as the 1928 crop—more or less tender and extensible. Plot 16 gave distinctly the best dough; it was fairly tough and of fairly good spring and elasticity. Plot 6 gave the tenderest dough.

The bread was better than that of the 1928 crop. The best loaf was 16. It had very good oven spring and volume, good outside appearance and crust with a good face. Its crumb was fairly close and even in grain, of good spring, pile and texture, and of a pale grey-white colour.

The loaves from Plots 2 and 8 were much poorer than the others. They had open coarse grain of crumb, and were of inferior spring, pile and texture.

As in the case of the 1928 crop, a test was also made in which each flour was blended with a sample of No. 1 Manitoba flour. The proportions used this time were 40 per cent. Broadbalk flour with 60 per cent. No. 1 Manitoba flour. Cob doughs were made and the procedure was the same as with the 1928 crop. All the blends made doughs of good body, fairly tough with good spring and extensibility, but all tended to fall off in body rather unduly as fermentation proceeded. Plots 3 and 6 became appreciably more tender, whilst 8 remained tougher than the others. On the tray at final proof 8, 3 and 2, in that order, showed the greatest flow.

Plots 16 and 9 gave the best loaves and were but little inferior to the all-Manitoba control which was a bold loaf of excellent oven spring and volume, and very good outside appearance and crust: it had good soft crumb, fairly close and even grain, and very good spring with a pale grey-white colour.

Plots 8 and 2 were much poorer than the others. Their crumb was open and coarse in grain, and of only fair spring, pile and texture, with a dull grey-white colour.

Table III shows the behaviour of the 1929 plots with respect to one another in dough and loaf, both when baked alone and when blended with the Manitoba flour.

DISCUSSION OF RESULTS.

Inspection of Table III, which shows the relative order of merit of plots of the respective crops, as revealed in early and in later baking tests, shows that no definite trend of improvement or of deterioration on storage can be assigned to any particular plot. For example, Plot 3 deteriorated markedly during storage in 1927, but improved relatively·

to the other plots in 1928. Plot 11 tended to fall off in 1926, but showed relative improvement in 1927.

Humphries(8), in the trials referred to, found that of the 1903 crop Plots 10 and 11 improved markedly after some months' storage, whereas Plots 2 and 3 did not significantly change. He found a general and fairly marked improvement on storage amongst members of the 1904 crop.

Table IV has been prepared for purposes of general comparison from the preceding table. It represents an attempt to "average" the results. of the earliest baking tests for each crop so as to show the general order of merit, with respect to dough and to loaf quality, of the respective plots, year by year.

Table IV. *General order of merit of the Broadbalk wheats with respect to dough and loaf qualities of several consecutive crops "averaged" as far as possible for each crop.*

1926 crop	1927 crop		1928 crop		1929 crop	
Loaves: generally poor	Doughs	Loaves: superior to those of 1926	Doughs	Loaves: very poor	Doughs	Loaves: superior to 1928
11	2	2, 6	6	6	16, 9	16
13	6, 7	7	3, 2, 5	8	7, 8	9
8	8	3, 8, 5	16	2, 3	2	7
16	3, 5	12	8	5, 10	3	6
12	9, 10, 11, 12	9, 10, 14	13, 14	11	6	3
10, 14, 9	13, 14, 16	11, 13, 16	10, 12	12, 13, 14, 16	—	8, 2
2	—	—	11	9	—	—
3, 5, 6, 20	—	—	7, 9	7	—	—

N.B. A horizontal line in the table between plots implies a marked drop in quality between the plots so separated.

It will be seen that it is not possible to draw any definite conclusions as to the effect of manurial treatment upon baking quality from the results for these four years, let alone from those for any one season only. The order of quality is essentially different every year. The best plot one year is the poorest another year and so on. There is no plot which may safely be said to maintain either a very high relative position or a very low one, and there is no question of any one plot retaining an unaltered quality whilst the others fluctuate.

Humphries(8) found that of the 1903 crop, which on the whole was poor, Plot 2 was the worst. He describes it as the worst flour he had ever seen. It evidently therefore rivalled Plots 7 and 9 of the 1929 crop, but it will be noticed that Plot 2 of the 1927 crop was relatively of excellent

quality. Humphries claims as his most important result the superiority of the wheat from the unmanured plot in both 1903 and 1904. It is evident, however, from Table IV that Plot 3, whilst not generally by any means the poorest plot, does not maintain a high position.

Plots 7, 10 and 11 were poor both in 1903 and 1904, and especially in the earlier year when climatic conditions were bad. Here again the harvests of a later decade bring no confirmation.

Without doubt the factors operating on wheat quality in the Broadbalk Field are so complex that adequate analysis may be based only on observations extending over a long period. It seems probable that varying climatic conditions play a great part and complicate any attempt to influence wheat quality directly by the simple application of fertilisers.

Although there thus seems no possibility, as far as the work already done on the Broadbalk wheats is concerned, of establishing the definite superiority of one type or dosage of manure as against another from the point of view of baking quality, it is perhaps worth while to make the following observations:

Apparently Plots 10, 11, 12, 13 and 14 associate together on the whole so that the presence or absence of given basic constituents, or of phosphates, in the fertiliser appears to have no discernible effect upon quality of the grain.

Plots 6 and 8 on the one hand and Plots 9 and 16 on the other tend to run together. When 16 and 9 are high in the order of relative merit then 6 and 8 are low and *vice versa*. Moreover, when 16 and 8 are good (or bad) then 9 and 6 also tend to be good (or bad). That is, when the nitrate plots are good the ammonia plots are poor and *vice versa*. This does not apply to the 1926 crop, however, when dose rather than type of manure appeared the more important, and 6 and 9 were both of low quality, and 8 and 16 both relatively higher in quality. When the ammonia plots are better and the nitrate poorer, the unmanured is up in quality and *vice versa*; except in 1926 when dose was important, and 3, the unmanured, was down in quality. No. 2, the dunged plot, tends to associate with 6, the plot with the smallest dose of ammonia.

It would be premature to speculate as to the causes which produce these apparent associations, and it will be necessary to collect more data before attempting to establish a differentiation between the action of nitrate and of ammoniacal fertilisers on the growing crop. One naturally has climatic influences most in mind and it is suggestive that the 1929 crop (when the apparent nitrate ammonia see-saw had turned in favour of the nitrate) followed an exceptionally warm dry summer. It is just possible

that under one set of climatic conditions the one type of manure tends to earlier assimilation or more rapid maturity than the other, with consequent differences in colloidal characteristics of the protein gel, but without affecting the total amount of nitrogenous material in the ripened grain.

It must be emphasised that the foregoing observations with respect to relative qualities of the wheats in no way imply corresponding differences in protein content. A comparison of Table II with Table IV shows that there is no correlation between the protein content of the Broadbalk flours and their baking quality. It is well known that it is necessary to distinguish between amount of protein and quality of protein in connection with "quality" in wheat.

In spite of the obscurity of the whole problem suggested by the observations recorded above, there are several lines of attack which readily suggest themselves. Two of these we have had the opportunity of exploring in a preliminary manner.

In the first place, it is necessary to explain that with many flours whose protein is relatively high in amount but of such physico-chemical properties that the flour is "weak," in the sense of producing soft doughs of low water absorption and poor stability, it is possible to influence baking quality favourably by appropriate physical treatment of the flour. Various methods of carrying out this treatment are known, two of which have been developed by the present writers[4] and patented in Great Britain and several other countries. We have found that the best method of carrying out the treatment on a small scale is to expose the flour in thin layers on trays to the action of a moving hot air belt of relative humidity above 60 per cent. and at a temperature of 160° F. for a period of about 15 minutes.

In 1929 portions of the flour from Plots 3 and 8 of the 1928 crop were heat treated in this way. The treated samples were baked together with the untreated.

As a result of the treatment both samples made tougher doughs of unimpaired elasticity and improved stability and handling quality generally. The bread from both untreated samples was very poor—as indeed had been found to be the case in the earlier bake on the 1928 crop as summarised in Table III. It was of only fair oven spring and volume, with open grained coarse crumb of only fair pile and texture and poor spring. Plot 3 was distinctly improved by the treatment, giving a loaf of fairly good oven spring, close and even grain of crumb which was of good pile and slightly better spring and colour than the untreated.

Plot 8 was very markedly improved by the treatment. The treated loaf was bold, of good oven spring and volume with crumb close and even in grain, of good spring, pile and texture, and of much better colour than the untreated.

The 1928 crop as a whole was of inferior baking quality. The doughs were tender and the bread very poor. It may be assumed, therefore, that the gluten was in such a physico-chemical condition that it implied "weakness" *per se*, but that it was capable of modification and improvement by suitable heat treatment. This being so, the flour of higher actual content of protein (Plot 8) would be expected to show the greater improvement.

In 1930 a portion of each sample of the 1929 crop was heat-treated and baked alongside the untreated flour.

Plot 2 showed marked all-round improvement as a result of the treatment. The loaf was bolder with better bloom of crust, the crumb was closer in grain and paler in colour. Plot 8 was appreciably improved though rather less markedly so than 2. Plots 3, 6 and 9 were slightly improved, whilst 7 and 16 were practically unaffected.

In the case of Plot 2 of the 1929 crop a further test was carried out in which 30 per cent. of the untreated flour and 40 per cent. of the treated were blended with 70 per cent. and with 60 per cent. respectively of untreated No. 1 Manitoba flour. Notwithstanding the additional amount of English flour carried, the treated blend gave a dough of markedly better body, and a better all-round cob loaf, than the untreated blend.

These results offer a definite suggestion that where the protein content has been increased as a result of manurial treatment, but is "weak" from the baking standpoint, it can be improved by certain physical treatment, whereas if it has been increased in amount and is satisfactory from the baking standpoint, or when low has not been increased in amount, the flour may not respond to physical treatment. The problem may be stated as follows: What are the conditions accompanying the production of increased protein content as a result of a certain definite manurial treatment which in one season confer colloidal properties upon the protein such as are associated with "weakness" and which can be modified by physical means in the direction of increased "strength," and which in another season form the colloid so that it is of satisfactory baking quality and less susceptible to improvement by physical means?

The other avenue of exploration is indicated by work carried out in the United States by J. Davidson [1, 2, 3] and collaborators. These workers studied the effect of the application of nitrogenous manures at various

stages during the growth of the wheat plant on the crop yield and nitrogen content of the grain. In general they found:

(1) The effectiveness of nitrates in increasing yields decreases progressively as the time of application approaches heading time.

(2) The effectiveness of nitrates in increasing protein content of the grain varies in the inverse sense to their effectiveness in increasing yield as far as heading time.

(3) After heading time (*i.e.* in the milk stage) the effect on yield was nil, and the effect on protein content was less than at heading time.

During 1928 and 1929 some preliminary trials were carried out at Rothamsted to test these conclusions. Four varieties of wheat were grown, viz. Yeoman II, Swedish Iron, Squarehead's Master and Million III. These were given respectively the following treatments:

(1) No top-dressing.

(2) No top-dressing.

(3) Sulphate of ammonia, 1 cwt. per acre applied early, *i.e.* at tillering.

(4) Muriate of ammonia, 1 cwt. per acre applied early, *i.e.* at tillering.

(5) Sulphate of ammonia, 1 cwt. per acre applied late, *i.e.* 8 weeks later.

(6) Muriate of ammonia, 1 cwt. per acre applied late, *i.e.* 8 weeks later.

(7) Sulphate of ammonia, early and late (*i.e.* a double dressing).

(8) Muriate of ammonia, early and late (*i.e.* a double dressing).

In each case the treatment was carried out on triplicate plots, each of one-fortieth acre: 96 plots in all.

Nitrogen determinations were carried out on each of the ninety-six samples of dressed grain received in each of the years 1928 and 1929. The results may be given shortly here: there was no significant increase in nitrogen content due to the manurial treatment in any instance.

Subsequently, certain of the triplicate samples of the 1929 crop were bulked so that milling and baking tests could be carried out. Four samples of straight-run flour were obtained from the Yeoman wheats, representing the following manurial treatments:

						Nitrogen content of the bulked sample of wheat
A 1.	Unmanured ...	...	...	...		1·47 %
A 2.	Sulphate of ammonia early			...		1·49 %
A 3.	„	„	late ...	...		1·49 %
A 4.	„	„	early and late			1·43 %

A similar series of samples was obtained of the Squarehead's Master series:

					Nitrogen content of the bulked sample of wheat
B 1.	Unmanured ...	...	...	...	1·50 %
B 2.	Sulphate of ammonia early		...	...	1·53 %
B 3.	,,	,,	late ...	...	1·52 %
B 4.	,,	,,	early and late		1·49 %

Extractions, i.e. flour yields, ranged between 71·5 and 73·3 per cent. of the wheat. The samples were milled during April and May, 1930, and baked during May and June.

Both series were baked on the multiple differential system developed in the writers' laboratories—in the first place alone, i.e. unblended, using different fermentation periods with 2 per cent. yeast; secondly, with 2 per cent. yeast and with a fixed fermentation period, but blended to the extent of 40 per cent. with 60 per cent. of each of two reference flours. These reference flours were straight-run commercial flours—a "strong" London flour and a relatively "weaker" north country flour respectively.

The Yeoman samples behaved alike in the dough. They made doughs of good body, which were claylike with fairly good stability but only fair spring; they tended to fall off unduly in body as fermentation proceeded. The bread, however, showed that the fermentation time of the samples, which was relatively long in all cases (i.e. 4 hours or more), lengthened slightly but progressively from A 1 to A 4, but that A 2 and A 3 gave distinctly better all-round loaves than A 1 and A 4. A 2 was slightly better than A 3; A 1 and A 4 were much alike. These results were substantially confirmed by those of the blending test.

The Squarehead's Master samples gave poor doughs and poor bread. The fermentation time was shorter than that of the Yeoman, but it was the same for all four samples: the best loaves in each case followed $3\frac{1}{4}$ hours' fermentation. There was no difference between the doughs of the four samples, but 3 gave distinctly the best bread and 1 the poorest; 2 was slightly better than 4.

A portion of each sample of flour was now heat-treated on the same lines as the Broadbalk samples. With the Yeoman samples all were distinctly improved by the heat treatment except A 3 which was only improved slightly. The effect of the heat treatment on fermentation time was slightly but consistently to increase it—less with the unmanured sample than with the manured—though 3 was less affected than 2 and 4.

With the Squarehead's Master samples there was no noticeable·

lengthening of fermentation time due to heat treatment, but the optimum loaves of samples B 1 and B 2 were markedly improved by the heat treatment, whereas those of B 3 and B 4 were slightly deteriorated.

Although it is not possible from the work described above to draw any definite conclusions as to the effect of the manurial treatment of wheat upon the baking quality of the flour, the work has revealed the extreme complexity of the problem and of the various factors, climatic and other, that appear to influence quality.

Moreover, two promising lines of investigation into the problem have been opened up. There is some evidence that the protein content of wheat may be materially increased by certain manurial treatment, although the precise nature and conditions of such treatment have so far eluded discovery. There is also evidence that the improving effect of certain types of heat treatment on "weak" English flours may be greater the greater the protein content of the flour. Further, the resulting improvement is more pronounced when the improved flour is blended with a "stronger" flour than when it is baked alone. If these provisional conclusions can be established as fact, they would have considerable economic importance in connection with wheat growing in this country. If the area under wheat is not to diminish still further, still more if the area is to be increased, it is essential that the wheat be grown, ground and consumed locally in order to avoid the heavy transport charges characteristic of this country. To bring this about it must be made possible for the country miller to use a greater proportion of English wheat in his grist. This can be done if the quality of the wheat can be improved in the two-fold manner indicated.

Summary.

In each of the four seasons 1926 to 1929 samples of certain of the Broadbalk wheats were milled, and the straight-run flours tested in the bakehouse.

The results given in the text show that the nitrogen contents of the wheats were increased consistently only by the heaviest nitrogenous treatments. The baking tests, however, showed that increased protein content was not necessarily accompanied by improved baking quality. In none of the crops was unsoundness of grain or inadequate gas production during fermentation a factor in the poor quality observed in many of the flours.

The baking results are summarised in Tables III and IV. The order

of the quality of the various plots was essentially different every year. No plot maintained either a high or a low relative position, and there was no question of any one plot retaining an unaltered quality whilst others fluctuated. No definite trend in degree of improvement or deterioration during storage of flour could be assigned to any particular plot or to the plots as a whole.

Evidently the factors operating on wheat quality in the Broadbalk field are so complex that adequate analysis may be based only on very extended observations, but certain tentative conclusions may be drawn from the results discussed. The presence or absence of given basic constituents, or of phosphates, in the fertiliser had no discernible effect upon the quality of the grain. Further, in years when nitrate fertiliser produced good results, the ammonium fertiliser gave poor effects and *vice versa*. The unmanured plot tended to be of better quality when the ammonia plots were better and the nitrate poorer, and *vice versa*. The dunged plot tended to associate with the plot receiving the lightest ammonia dressing.

It is possible that climatic influences determine the effect of one type of manure as opposed to another in producing favourable or unfavourable colloidal characteristics in the protein gel of the ripened grain without affecting the total amount of nitrogenous material present.

The Broadbalk flours appear to vary in the extent of their response to a process of heat treatment, which, with many flours, is known to have the effect of modifying the physico-chemical condition of the gluten favourably (*i.e.* in the direction of improved baking quality). Experiment tended to show that where protein content has been increased as a result of manurial treatment, but the flour is "weak" from the baking standpoint, improvement follows the physical treatment, whereas if the protein has been increased in amount and is satisfactory in quality, or when low has not been increased in amount, the flour may not respond to physical treatment. In the case of one flour where improvement following heat treatment was marked, the effect of the treatment was even more marked when baking tests were carried out on blends of the untreated and treated flour with an untreated "strong" No. 1 N. Manitoba flour.

Flours were examined also from wheats grown at Rothamsted during 1929 to test the effect of the application of nitrogenous manure (in the form of ammonium salts) at an early and at a late stage of plant development respectively. The growing trials were comprehensively planned but no significant increase in nitrogen content due to manurial treatment was found in any instance. The doses, however, were less than those used on ·

the Broadbalk plots and were perhaps more in accord with commercial practice. The results of the baking quality comparisons were not conclusive, though with both Yeoman and Squarehead's Master certain of the manured samples had appreciably better quality than the unmanured.

We desire to express our thanks to our colleagues, Mr R. H. Carter and Dr P. Halton, for the large amount of work involved in providing us with the analytical and gas production data. Our thanks are due in a special degree to Sir John Russell, F.R.S., for so readily giving us the wheats and for modifying a Rothamsted field experiment in 1928 and in 1929 to enable us to carry out the preliminary study of the effect of nitrogenous manuring on protein content described in the latter part of the paper. The baking tests were carried out by Mr W. E. Spencer, baker to the Research Association, whose skilful help we wish to acknowledge.

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THE NATURE OF SOIL ORGANIC MATTER AS SHOWN BY THE ATTACK OF HYDROGEN PEROXIDE.

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(With Two Text-figures.)

THE organic matter of soil has received the attention of numerous investigators from time to time, and a considerable body of information has now accumulated, more especially in connection with its physical properties. When considered, however, from a purely chemical stand-point, it is remarkable how little definite knowledge has been gained with regard to its composition. No doubt its admittedly complex character is responsible for this. Most investigations have centred round a par-ticular fraction of the organic matter, viz. that portion soluble in alkalies, but the chemical identity of this fraction is still in question, as varying quantitative results are obtained depending on the nature and con-centration of the alkali and on the temperature and length of extraction. From this fraction a large number of individual organic compounds have been isolated and identified by Shorey[1], but it is questionable if any of these compounds exist in the soil as such.

Recent investigators, particularly Waksman and his associates, have adopted a new line of attack in order to get an insight into the general chemical composition of organic matter as a whole. The idea underlying their method is to separate organic matter into various broad categories or complexes which have a similar chemical nature by means of suitable solvents. These are used in a definite order for the elimination of the various complexes.

In view of the bearing of the present investigation on the results obtained by Waksman and Stevens[2], examples of the results obtained by them for mineral soils are given below in an excerpt from their recent paper.

"The two largest groups of chemical complexes found in the soil organic matter are the 'lignin-humus' complex (soil lignin or 'soil humus') and the nitrogenous compounds. These two make up 71 to 80 per cent. of the total organic matter of the soil and an even larger

amount of that part of the organic matter which is accounted for in these analyses. The high carbon content of the soil organic matter

Proximate chemical composition of soil organic matter.
(On the basis of the total organic matter (C × 1·72).*)*

Soil No.	Ether-soluble material (%)	Alcohol-soluble material (%)	Hemi-celluloses (%)	Cellu-loses (%)	Lignin-humus complex (%)	Organic nitro-genous complexes (%)	Sum of con-stituents accounted for (%)
4	3·56	0·58	5·44	3·55	43·37	33·78	90·28
16	0·80	0·82	5·53	4·12	41·87	37·35	90·49

(usually taken as 58 per cent.) is due to the predominance of the lignins, lignin-like complexes, and lignin derivatives with a high carbon content (62 to 64 per cent.). The more or less definite relation between the 'lignin-humus' complex and the organic-nitrogenous complexes accounts for the more or less constant ratio between the carbon and nitrogen in the soil organic matter.

The close correlation between these two groups of complexes in the soil organic matter may be a matter of chance, or it may be due to the actual formation of a chemical compound between the two whereby they are made more resistant to the action of the micro-organisms."

The bulk of the organic matter of the soil would seem, therefore, to consist of a lignin-humus complex and an organic nitrogenous complex and, as suggested in the above excerpt, these two complexes may be in chemical combination since they persist in the soil and consequently must be resistant to the action of micro-organisms. If the nitrogenous complex were present in the soil as a protein it would, of course, be readily attacked by micro-organisms.

In a contribution to this *Journal*(3), the writer demonstrated that weak hydrogen peroxide (3 per cent.) removed from the soil an oxidisable complex which accounted for about 80 per cent. of its total carbon and nitrogen. When a stronger peroxide (6 per cent.) was used, this complex together with a small amount of a non-nitrogenous complex was removed, leaving unattacked in the residue all the nitrogen not previously accounted for and the remainder of the carbon. As the carbon-nitrogen ratio of the oxidisable complex is approximately 10 : 1, it cannot be a compound of the nature of a protein but may be a complex consisting of a protein in combination with a non-nitrogenous complex of high carbon content.

If this latter complex could be identified as being a substance of the nature of lignin, *i.e.* having a carbon content of about 62 per cent.,

support would be forthcoming for the view suggested by Waksman that the close correlation between the lignin-humus complex and the organic nitrogenous complexes may be due to the actual formation of a chemical compound between the two.

We may arrive at an idea of the nature of the oxidisable complex by making use of two assumptions. In the first place, the factor 1·72 commonly used for deriving soil organic matter from organic carbon may be used in calculating the amount of oxidisable complex. This assumes that the carbon content is the same as that generally taken for soil organic matter as a whole. Secondly, the nitrogen of the oxidisable complex may be calculated to protein by using the factor 6·25, thus assuming that it is of normal protein character. Subtracting the protein from the oxidisable complex the amount of non-nitrogenous (lignin-humus) complex is given and its carbon content may be calculated.

An example of the calculation in the case of an Anglesey soil is as follows:

Carbon 4·59 per cent. and *nitrogen* 0·449 per cent. oxidised by 3 per cent. peroxide.

Oxidisable complex: 4·59 × 1·72 = 7·89 per cent.

Protein complex: 0·449 × 6·25 = 2·81, which contains 1·41 per cent. carbon (assuming that protein contains 50·0 per cent. carbon).

Lignin-humus complex: 7·89 − 2·81 = 5·08 per cent., which contains 4·59 − 1·41 = 3·18 per cent. carbon.

Therefore, percentage carbon in *lignin-humus complex* is

$$\frac{3 \cdot 18 \times 100}{5 \cdot 08} = 62 \cdot 6.$$

The results obtained in this way for twenty-six soils of varying origin are given in Table I.

The figures in the extreme right-hand column of Table I are remarkably constant. They suggest that the organic matter of soils in general consists largely of an oxidisable complex, which is composed of a protein compound intimately associated or in direct combination with a lignin-like substance. *This, in effect, implies that the bulk of organic matter of all soils has the same composition*, a view which is not surprising when one considers that the original plant materials from which all soil organic matter is derived have approximately the same composition in all parts of the world.

The only reason why one might expect an organic matter which varied in composition from place to place is that widely varying temperature and moisture conditions might lead to the development of different

Table I.

Soil	Total		Oxidised by 3% hydrogen peroxide			Oxidisable complex		Protein complex of oxidisable complex (%)	Carbon in lignin-humus complex (%)
	C (%)	N (%)	C (%)	N (%)	Oxidisable complex C × 1.72	Protein complex in soil (%)	Lignin-humus complex in soil (%)		
Broadbalk, F.Y.M. Rothamsted	2·70	0·26	2·09	0·219	3·59	1·38	2·21	38·4	63·3
Broadbalk, no manure, Rothamsted	1·06	0·12	0·59	0·076	1·01	0·48	0·53	47·5	66·0
Barnfield, F.Y.M. Rothamsted	2·85	0·28	2·24	0·227	3·85	1·42	2·43	36·9	63·0
Barnfield, no manure, Rothamsted	0·71	0·11	0·27	0·046	0·46	0·29	0·17	63·0	70·6
Herefordshire, 139	1·62	0·19	1·30	0·144	2·24	0·90	1·34	40·2	63·4
Herefordshire, 331	1·32	0·15	0·87	0·099	1·50	0·62	0·88	41·3	63·6
Herefordshire, 92	2·42	0·23	2·05	0·182	3·53	1·14	2·39	32·3	61·9
Llysfasi, A. Denbighshire	2·33	0·24	1·75	0·193	3·01	1·21	1·80	40·2	63·3
Black soil, Kenya	2·18	0·14	0·55	0·043	0·95	0·27	0·68	28·4	60·3
Black clay, Trinidad	2·16	0·21	1·16	0·115	2·00	0·72	1·28	36·0	62·5
Black turf soil, Transvaal	1·41	0·10	0·62	0·048	1·07	0·30	0·77	28·0	61·0
Stackyard, F.Y.M. Woburn	1·51	0·15	1·29	0·128	2·22	0·80	1·42	36·0	62·1
Stackyard, no manure, Woburn	0·90	0·10	0·78	0·075	1·34	0·47	0·87	35·1	62·1
Herefordshire, 26	1·71	0·19	1·37	0·146	2·36	0·91	1·45	38·6	62·8
Aber, T. 12	3·26	0·39	2·68	0·328	4·61	2·05	2·56	44·4	64·4
Aber, 2	3·97	0·40	3·54	0·344	6·09	2·15	3·94	35·3	62·4
Aber, 6	2·78	0·29	2·34	0·228	4·02	1·43	2·59	35·6	62·5
Aber, B. 12	2·71	0·31	2·26	0·255	3·89	1·59	2·30	40·9	63·5
Madryn 2, Carnarvonshire	4·31	0·39	3·82	0·346	6·57	2·16	4·41	32·9	62·1
Bodrwyn, Anglesey	5·07	0·51	4·59	0·449	7·89	2·81	5·08	35·6	62·6
Bangor	6·80	0·59	5·58	0·522	9·60	3·26	6·34	34·0	62·3
Llediwigan, Anglesey	4·08	0·43	3·39	0·344	5·83	2·15	3·68	36·9	62·8
Padi soil, Siam, B.A.S. 9	6·28	0·54	5·82	0·434	10·01	2·71	7·30	27·0	61·1
Padi soil, Siam, B.A.S. 3	1·63	0·18	1·44	0·113	2·48	0·71	1·77	28·6	61·0
Red clay, Trinidad	1·33	0·25	1·14	0·112	1·96	0·70	1·26	35·7	62·6
Black loam soil, Transvaal	1·87	0·13	1·30	0·086	2·24	0·54	1·70	24·1	60·6

types of micro-organisms which would ultimately be responsible for the decomposition of the organic material added to the soil. Even if this were so, it is the synthesised microbial substance which, in the view of some writers, persists in the soil as organic matter that would be likely to vary in composition and not the material which has resisted the action of micro-organisms. This latter substance is admitted by most investigators to be carbohydrate in character—high in carbon content—whereas the synthesised material is a nitrogenous compound, and it is therefore in the composition of this material that a difference might be expected which would be reflected in the nitrogen content of the soil organic matter.

It has been shown by the writer[4] that in the case of British soils where climatic conditions are broadly the same, the proportion of nitrogen to carbon in the organic matter is approximately constant, whereas in tropical countries the proportion of nitrogen to carbon in the organic matter of soils varies and is widely different from that found for British soils.

Potter and Snyder[5] have shown that organic matter as distributed by the Van Slyke method is essentially the same in soils differently treated and in different soil types. The nature of the nitrogen distribution in the soil organic complexes is different from that of the nitrogen distribution in proteins of plant and animal origin. Arnold and Page[6] have shown that there is a marked similarity in the character of the soil organic matter of certain Rothamsted plots, so far as solubility in weak caustic soda is concerned, in spite of the fact that these different plots had received organic matter of widely different nature and composition.

Before accepting the conclusions drawn from the results in Table I, which seem to agree in the main with the conclusions of other investigators, the question arises as to whether the assumptions on which the calculations from the experimental data are based are justifiable. Waksman, in calculating his results for the proximate analyses of organic matter, makes use of the factor 1·72 for calculating total organic matter from total carbon, and 6·25 for converting nitrogen into organic nitrogenous complex, and further assumes that the carbon and nitrogen in the residual soil after treatment with various reagents and finally with 80 per cent. sulphuric acid are present in the form of lignin and protein and computes the amount of the former by assuming its carbon content to be 62 per cent.

Figures obtained on the basis of so many assumptions which are purely tentative can only be regarded at the best as approximations.

For this reason the writer decided to carry out the following investigation, dispensing with all such assumptions.

Previous work on the effect of hydrogen peroxide on soil organic matter showed that a large percentage was readily oxidised by dilute peroxide (3 per cent.), but a portion resisted the attack of even large quantities of 6 per cent. peroxide. It was thought that useful information might be gained by working with still weaker peroxide and with peroxide stronger than 6 per cent. Further, it was felt that much more definite knowledge as to the nature of organic matter would be obtained by *determining experimentally the weight of organic matter oxidised by each concentration of peroxide.* (It will be recalled that for the calculations in Table I, the amount of carbon oxidised was multiplied by the factor 1·72 in order to get the weight of the oxidisable complex.)

Knowing the loss of organic matter due to oxidation by hydrogen peroxide and the percentages of oxidisable carbon and nitrogen in the soil, the percentages of carbon and nitrogen in the oxidisable complex can be calculated.

<div align="center">EXPERIMENTAL.</div>

The method is as follows for each concentration of peroxide used:

Equal amounts of air-dried soil (100 mesh sample) containing approximately 0·08 gm. of organic carbon are weighed into two 400 c.c. beakers from a weighing bottle. At the same time duplicate samples are weighed out for determination of moisture as well as duplicate samples for total organic carbon and nitrogen. Sixty c.c. of peroxide of the desired concentration are measured into each beaker, heated gently at first and afterwards boiled down to small bulk, when about 40 c.c. of water are added and again boiled down to small bulk. The contents of the beakers are allowed to settle (usually overnight) and then filtered through small weighed hardened filter papers of known moisture content and washed thoroughly with hot water. One of the residues including the paper is transferred to a tared weighing bottle; the other residue is washed off the paper into a large tared weighing bottle, and the paper to which some soil usually clings is put into another small tared weighing bottle. All the weighing bottles are now transferred to an electric oven kept at 104° C. and dried to constant weight and weighed. The filtrate in each case is evaporated to dryness in a weighed, ignited dish and afterwards ignited in a muffle furnace and weighed. The latter procedure is necessary in order to get a correction for the loss of mineral matter from the soil due to the peroxide treatment. A correction is also made for the ignited

mineral matter found to be present in the peroxide used. All the data necessary for the determination of the amount of organic matter removed from the soil by oxidation (stated as a percentage of the total dry soil) are thus obtained.

The residue in the large weighing bottle is now transferred to a mortar, ground, and transferred to a Kjeldahl flask, and the carbon and nitrogen percentages determined. Since the percentages of the total carbon and nitrogen are known, the percentages of carbon and nitrogen oxidised by peroxide can be calculated and are here stated as percentages of the original soil dry at 104° C.

In Table II are given the results for two carbonate-free Welsh soils.

The following conclusions may be drawn from the data in Table II:

A. An oxidisable complex has been progressively decomposed by peroxide of 0·21 per cent., 0·42 per cent. and 0·84 per cent. concentration in the case of both soils.

B. The percentage composition of the oxidisable complex in terms of carbon and nitrogen is 52·8 per cent. and 5·3 per cent. respectively in the Madryn, and 52·4 per cent. and 5·3 per cent. respectively in the Bodrwyn soil.

C. A 1·26 per cent. solution of hydrogen peroxide is sufficient to remove completely the oxidisable complex as indicated by the same amount of nitrogen being present in the residual soils after treatment with higher concentrations of peroxide.

D. The oxidisable complex is constant in composition in so far, at any rate, as its percentages of carbon and nitrogen are concerned. Increasing amounts of this complex are removed by increasing concentrations of peroxide until it has been removed completely.

E. By increasing the concentration of peroxide beyond the point at which all the oxidisable complex has been decomposed, an increasing quantity of a non-nitrogenous complex is attacked.

F. Twelve per cent. hydrogen peroxide, even in repeated applications, fails to oxidise completely all the carbon of the organic matter, and does not remove any more nitrogen than 1·26 per cent. peroxide.

G. The C/N ratio of the oxidisable complex is 10 : 1 in the case of each soil.

The outstanding feature of the above results is the constant composition of the oxidisable complex and the ease with which it is oxidised. Its amount in the soil cannot be expressed as a percentage of the total organic matter, since this is not known and cannot be calculated unless an assumption as to its composition is made. An idea of its

Table II. *Effect of varying strengths of peroxide. Soils—Bodrwyn and Madryn.*

Peroxide (%)	Total C(%)	Total N(%)	C/N ratio	Residual C(%)	Residual N(%)	C/N ratio	Oxidised C(%)	Oxidised N(%)	C/N ratio	Total oxidised C(%)	Total oxidised N(%)	Loss of organic matter on oxidation (%)	Oxidisable complex C(%)	Oxidisable complex N(%)
							Soil—Bodrwyn							
0·21	5·59	0·539	10·4	3·20	0·312	10·1	2·39	0·227	10·5	42·8	42·1	4·58	52·2	5·0
0·42	5·59	0·539	10·4	2·00	0·171	11·7	3·59	0·368	9·8	64·2	68·3	6·80	52·8	5·4
0·84	5·59	0·539	10·4	1·22	0·091	13·4	4·37	0·448	9·8	78·2	83·1	8·37	52·2	5·4
1·26	5·59	0·539	10·4	0·75	0·075	10·0	4·84	0·464	10·4	86·6	86·1	8·97	53·9	5·3*
1·68	5·59	0·539	10·4	0·70	0·075	9·3	4·89	0·464	10·5	87·6	86·1	8·72	56·1	5·3*
2·50	5·59	0·539	10·4	0·56	0·074	7·6	5·03	0·465	10·8	90·0	86·3	9·00	55·9	5·2*
3·75	5·59	0·539	10·4	0·49	0·076	6·4	5·10	0·463	11·0	91·2	86·0	8·85	57·6	5·2*
5·00	5·59	0·539	10·4	0·43	0·076	5·7	5·16	0·463	11·1	92·3	86·0	8·79	58·7	5·2*
12·00	5·59	0·539	10·4	0·33	0·078	4·2	5·26	0·461	11·4	94·1	85·5	—	—	—
							Soil—Madryn							
0·21	4·16	0·385	10·8	2·18	0·199	11·0	1·98	0·186	10·6	47·6	51·7	3·64	54·4	5·2
0·42	4·16	0·385	10·8	1·51	0·112	13·5	2·65	0·273	9·7	63·7	70·9	5·15	51·5	5·3
0·84	4·16	0·385	10·8	0·87	0·057	15·3	3·29	0·328	10·0	79·1	85·2	6·25	52·6	5·3
1·68	4·16	0·385	10·8	0·55	0·045	12·2	3·61	0·340	10·6	86·7	88·3	6·39	56·5	5·3*
2·50	4·16	0·385	10·8	0·48	0·041	11·7	3·68	0·344	10·7	88·5	89·3	6·40	57·5	5·4*
3·75	4·16	0·385	10·8	0·40	0·042	9·5	3·76	0·343	11·0	90·4	89·1	6·59	57·1	5·2*
5·00	4·16	0·385	10·8	0·35	0·044	8·0	3·81	0·341	11·2	91·6	88·6	6·38	60·7	5·4*
12·00	4·16	0·385	10·8	0·30	0·045	6·7	3·86	0·340	11·4	92·8	88·3	—	—	—

Average (Bodrwyn): C (%) = 52·4, N (%) = 5·3, C/N = 9·95

Average (Madryn): C (%) = 52·8, N (%) = 5·3, C/N = 10·0

* These figures include some of the non-nitrogenous complex.

It was intended to work with 0·25, 0·50, 1, 2, 3 %, etc. solutions, but it was not until after these had been prepared that the original peroxide was found to be 5 % instead of the normal 6 %.

amount can be best expressed in terms of the total carbon and nitrogen of the soil. In the Bodrwyn soil *at least* 78·2 per cent. of the total carbon and 83·1 per cent. of the total nitrogen are present in the oxidisable complex. The figures for the Madryn soil are seen to be very similar. A further 15·9 per cent. of the total carbon of the Bodrwyn soil and 13·7 per cent. of the Madryn soil are present in a carbon compound of an unknown nature.

Since the oxidisable complex decomposed by very dilute peroxide has the same composition—expressed in terms of carbon and nitrogen— in the case of the two carbonate-free soils examined, a number of other soils was treated with 1 per cent. peroxide in order to ascertain if the composition of this complex is the same for soils *in general*. This strength was used in order to ensure that nothing but the oxidisable complex was attacked.

Results are given in Table III.

The composition of the oxidisable complex is approximately the same as that given in Table II, except in the case of soil Aber 3, where it is decidedly different. It was discovered that this soil contains calcium carbonate. Two soils containing large amounts of calcium carbonate were accordingly examined in an exhaustive manner.

Results are presented in Table IV.

As in the case of soils recorded in Table II, here again the percentage composition of the oxidisable complex is constant for each soil and is progressively oxidised. Smaller fractions of this complex are oxidised by equivalent concentrations of peroxide than in non-carbonate soils. In the Broadbalk soil it has a higher nitrogen content as well as a higher carbon content than in the non-carbonate soils, but its C/N ratio is the same, viz. 10 : 1. In the Llysfasi soil, however, the nitrogen content is higher but the carbon content is lower than in the case of non-carbonate soils, and its C/N ratio is 8·4 : 1. It should be mentioned here that there may be an error in the determination of the percentage "loss of organic matter on oxidation" in the carbonate soils for the reason that there is an appreciably greater residue of ignited material derived from the filtrate (obtained after filtering the peroxided soil) in carbonate than in non-carbonate soils. Calcium compounds are here weighed in the form of oxide or carbonate, and it is not clear in what form or in what proportion they are in the original soil or in the ignited residue. The composition of the oxidisable complex in the Llysfasi soil, viz. 50·5 per cent. carbon and 6·0 per cent. nitrogen, indicates that, although there may be an error which would have the effect of throwing the

Table III.

Soil	Peroxide (%)	Total C (%)	Total N (%)	C/N ratio	Residual C (%)	Residual N (%)	C/N ratio	Oxidised C (%)	Oxidised N (%)	C/N ratio	Total oxidised C (%)	Total oxidised N (%)	Loss of organic matter on oxidation (%)	Oxidisable complex C (%)	Oxidisable complex N (%)
Aber, 14	1·0	2·56	0·295	8·7	0·56	0·081	6·9	2·00	0·214	9·3	78·1	72·5	3·84	52·1	5·6
Aber, 16	1·0	3·25	0·342	9·5	0·83	0·096	8·6	2·42	0·246	9·8	74·5	71·9	4·61	52·5	5·3
Stackyard, F.Y.M.	1·0	1·73	0·180	9·6	0·30	0·036	8·3	1·43	0·144	9·9	82·7	80·1	2·66	53·8	5·4
Aber, 3	1·0	3·17	0·316	10·0	0·48	0·061	7·9	2·69	0·255	10·5	84·9	80·7	4·41	61·0	5·8

Table IV. Effect of varying strengths of peroxide. Soils—Llysfasi and Broadbalk, F.Y.M.

Peroxide (%)	Total C (%)	Total N (%)	C/N ratio	Residual C (%)	Residual N (%)	C/N ratio	Oxidised C (%)	Oxidised N (%)	C/N ratio	Total oxidised C (%)	Total oxidised N (%)	Loss of organic matter on oxidation (%)	Oxidisable complex C (%)	Oxidisable complex N (%)
					Soil—Llysfasi									
0·25	2·40	0·261	9·2	1·89	0·196	9·6	0·51	0·065	7·8	21·3	24·9	1·07	47·7	6·1
0·50	2·40	0·261	9·2	1·43	0·150	9·5	0·97	0·111	8·7	40·4	42·5	1·81	53·6	6·1
1·00	2·40	0·261	9·2	0·88	0·081	10·9	1·52	0·180	8·4	63·3	69·0	3·03	50·2	5·9*
2·00	2·40	0·261	9·2	0·53	0·051	10·4	1·87	0·210	8·9	77·9	80·5	3·40	55·0	6·2*
5·00	2·40	0·261	9·2	0·37	0·049	7·6	2·03	0·212	9·6	84·6	81·2	3·44	59·0	6·2*
12·00 (60 c.c.)	2·40	0·261	9·2	0·32	0·052	6·1	2·08	0·209	10·0	86·7	80·1	3·44	—	—
12·00 (120 c.c.)	2·40	0·261	9·2	0·23	0·048	4·8	2·17	0·213	10·2	90·4	81·6	—	—	—
					Soil—Broadbalk, F.Y.M.									
0·25	2·84	0·272	10·4	2·23	0·209	10·7	0·61	0·063	9·7	21·5	23·2	1·08	56·5	5·8
0·50	2·84	0·272	10·4	1·82	0·175	10·5	1·02	0·099	10·3	35·9	36·4	1·70	60·0	5·8
1·00	2·84	0·272	10·4	1·13	0·095	11·9	1·71	0·177	9·7	60·2	65·1	3·00	57·0	5·9
3·00	2·84	0·272	10·4	0·53	0·045	11·8	2·31	0·227	10·2	81·3	83·5	3·78	61·1	6·0
5·00	2·84	0·272	10·4	0·39	0·032	12·2	2·45	0·240	10·6	86·3	88·2	3·79	64·6	6·3*
12·00 (60 c.c.)	2·84	0·272	10·4	0·23	0·025	9·2	2·61	0·247	10·6	91·9	90·8	4·34	—	—
12·00 (120 c.c.)	2·84	0·272	10·4	0·22	0·026	8·5	2·62	0·246	10·7	92·3	90·4	4·30	—	—

Average (Llysfasi): C (%) 50·5, N (%) 6·0, C/N = 8·4.

Average (Broadbalk): C (%) 58·7, N (%) 5·9, C/N = 10·0.

* These figures include some of the non-nitrogenous complex.
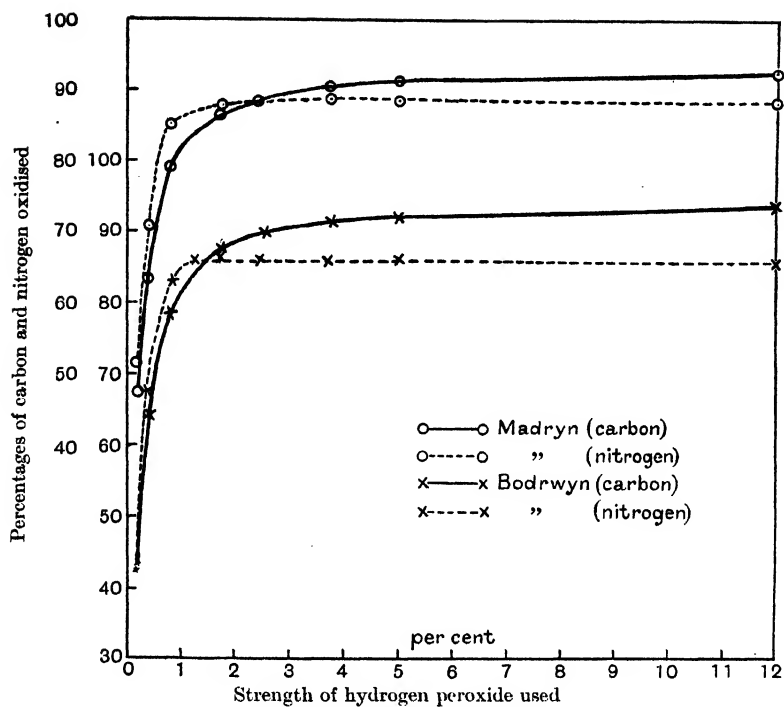
Fig. 1. Effect of hydrogen peroxide on soil organic matter.
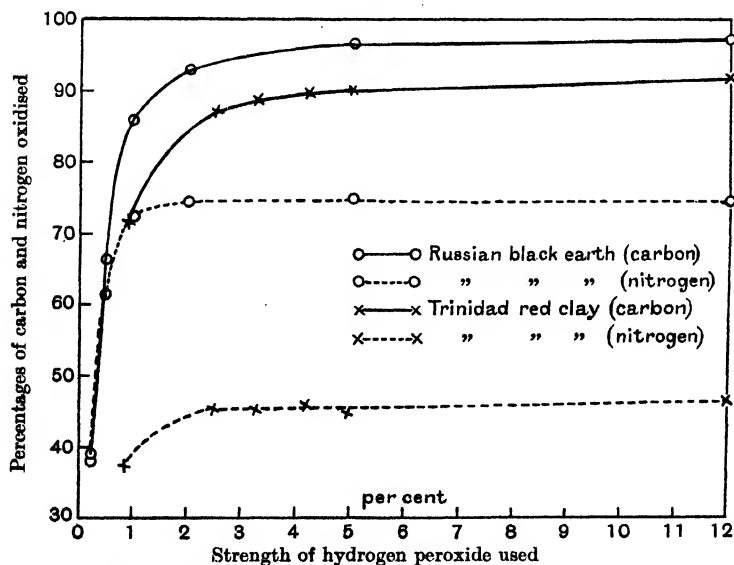
Fig. 2. Effect of hydrogen peroxide on soil organic matter.

percentages of carbon and nitrogen either too high or too low, *this complex has not the same composition in all soils*, and further that its C/N ratio is the factor which may determine the C/N ratio of the original soil. In order to test the latter suggestion and to gain further knowledge of the composition of the oxidisable complex in a variety of soil types, four foreign soils of varying C/N ratios were employed.

Results for these are given in Tables V and VI.

Examples of curves for the percentages of the total carbon and nitrogen removed from the soil by varying strengths of peroxide are given in Figs. 1 and 2.

The results in Tables V and VI demonstrate that in foreign soils as in British soils there is an oxidisable complex specific to the soil itself but constant in composition as shown by the fact that a substance of the same composition is progressively oxidised by various concentrations of peroxide. This complex in the Siam soil is extraordinarily easily oxidised (practically 50 per cent. of the total carbon and nitrogen being oxidised by 0·25 per cent. peroxide). Its composition (carbon 55·5 per cent. and nitrogen 4·45 per cent.) is similar to the Russian black earth (carbon 53·3 per cent. and nitrogen 4·3 per cent.) and to the Doleritic Natal soil (carbon 55·2 per cent. and nitrogen 4·1 per cent.). The wide C/N ratios of these complexes which form a big proportion of the total organic matter seem without doubt to account for the correspondingly wide C/N ratios of the original soils.

The Trinidad soil deserves special mention, being outstanding in many respects. The original soil has a very low C/N ratio—one of the lowest encountered by the writer. Dilute peroxide decomposed an oxidisable complex which is very similar to that of other soils. One might reasonably have expected this complex to have been exceedingly high in nitrogen—out of all proportion to that found in other soils. This is not so owing to the low percentage of the total nitrogen oxidised in the soil. Only about 40 per cent. of the total nitrogen is present in the oxidisable complex, whereas in all other soils examined there is at least 70 per cent.

The fact that an oxidisable complex approximately similar in composition to that of all other soils has been decomposed in this particular soil seems to the writer exceedingly strong confirmatory proof that there is in reality such a complex present in all soils.

Table V. *Effect of varying strengths of peroxide. Soils—Siam 9 and Russian Black Earth.*

Peroxide (%)	Total		C/N ratio	Residual		C/N ratio	Oxidised		C/N ratio	Total oxidised		Loss of organic matter on oxidation (%)	Oxidisable complex		Average
	C (%)	N (%)		C (%)	N (%)		C (%)	N (%)		C (%)	N (%)		C (%)	N (%)	
							Soil—Siam 9								
0·25	6·53	0·565	11·6	(illeg.)	0·303	11·4	3·15	0·262	12·0	48·2	46·4	5·65	55·7	4·6	C (%) 55·5
0·50	6·53	0·565	11·6	(illeg.)	0·189	9·0	4·82	0·376	12·8	73·8	66·5	8·73	55·2	4·3	N (%) 4·5
1·00	6·53	0·565	11·6	0·??	0·131	4·5	5·94	0·434	13·6	91·0	75·0	10·10	58·8	4·3*	C/N = 12·5
2·00	6·53	0·565	11·6	0·28	0·131	2·1	6·25	0·434	14·4	95·7	75·0	10·41	—	—	
5·00	6·53	0·565	11·6	0·21	0·124	1·7	6·32	0·441	14·3	96·8	78·0	9·8	—	—	
12·00 (60 c.c.)	6·53	0·565	11·6	0·24	0·115	2·1	6·29	0·454	14·0	96·3	79·6	9·5	—	—	
12·00 (120 c.c.)	6·53	0·565	11·6	0·22	0·110	2·0	6·31	0·455	13·9	96·6	80·5	9·8	—	—	
							Soil—Russian black earth								
0·25	3·05	0·260	11·7	1·85	0·161	11·5	1·20	0·099	12·1	39·3	38·1	2·23	53·8	4·4	C (%) 53·3
0·50	3·05	0·260	11·7	1·02	0·099	10·3	2·03	0·161	12·6	66·6	61·9	3·85	52·7	4·2	N (%) 4·3
1·00	3·05	0·260	11·7	0·42	0·071	5·9	2·63	0·189	13·9	86·2	72·7	4·62	56·9	4·1*	C/N = 12·4
2·00	3·05	0·260	11·7	0·21	0·066	3·2	2·84	0·194	14·6	93·1	74·6	4·70	60·4	4·1*	
5·00	3·05	0·260	11·7	0·11	0·065	1·7	2·94	0·195	15·1	96·4	75·0	4·77	61·6	4·1*	
12·00 (60 c.c.)	3·05	0·260	11·7	0·08	0·066	1·2	2·97	0·194	15·3	96·4	74·6	4·83	—	—	
12·00 (120 c.c.)	3·05	0·260	11·7	0·07	0·072	1·0	2·98	0·188	15·9	97·7	72·3	4·86	—	—	

* These figures include some of the non-nitrogenous complex.

Table VI. *Effect of varying strengths of peroxide. Soils—Doleritic Red Loam (Natal) and Trinidad Red Clay.*

Peroxide (%)	Total C(%)	Total N(%)	C/N ratio	Residual C(%)	Residual N(%)	C/N ratio	Oxidised C(%)	Oxidised N(%)	C/N ratio	Total oxidised C(%)	Total oxidised N(%)	Loss of organic matter on oxidation (%)	Oxidisable complex C(%)	Oxidisable complex N(%)
						Soil—Doleritic red loam (Natal)								
0·50	4·44	0·321	13·8	3·12	0·217	14·4	1·32	0·104	12·7	29·7	32·4	2·39	55·2	4·4
1·00	4·44	0·321	13·8	1·61	0·114	14·1	2·83	0·207	13·7	63·7	64·5	5·17	54·7	4·0
2·00	4·44	0·321	13·8	0·71	0·052	13·7	3·73	0·269	13·9	84·0	83·8	6·70	55·7	4·0
5·00	4·44	0·321	13·8	0·41	0·047	8·7	4·03	0·274	14·7	90·8	85·4	6·70	60·1	4·1*
12·00 (60 c.c.)	4·44	0·321	13·8	0·26	0·045	5·8	4·18	0·276	15·1	94·1	86·0	7·06	—	—
12·00 (120 c.c.)	4·44	0·321	13·8	0·20	0·042	4·8	4·24	0·279	15·1	95·5	86·9	7·04	—	—
						Soil—Trinidad red clay								
0·84	1·32	0·268	4·9	0·38	0·168	2·3	0·94	0·100	9·4	71·2	37·3	1·79	52·5	5·6
2·50	1·32	0·268	4·9	0·17	0·145	1·2	1·15	0·123	9·4	87·1	45·9	1·99	57·8	6·2*
3·33	1·32	0·268	4·9	0·15	0·146	1·0	1·17	0·122	9·6	88·6	45·5	2·01	58·2	6·1*
4·20	1·32	0·268	4·9	0·14	0·145	1·0	1·18	0·123	9·6	89·4	45·9	2·01	58·7	6·2*
5·00	1·32	0·268	4·9	0·13	0·149	0·9	1·19	0·119	10·0	90·1	44·4	2·40	—	—
12·00 (60 c.c.)	1·32	0·268	4·9	0·10	0·142	0·7	1·22	0·126	9·7	92·4	47·0	2·20	—	—
12·00 (90 c.c.)	1·32	0·268	4·9	0·09	0·138	0·7	1·23	0·130	9·4	93·2	48·5	2·12	—	—

Average (Doleritic red loam): C (%) 55·2, N (%) 4·1, C/N = 13·4

Average (Trinidad red clay): C (%) 52·5, N (%) 5·6, C/N = 9·4

* These figures include some of the non-nitrogenous complex.

NOTE.—It was impossible to filter the Trinidad soil after treatment with lower concentrations of peroxide.

GENERAL DISCUSSION.

A critical examination of the experimental results of this investigation indicates a conception of soil organic matter as a whole which has not hitherto been proposed.

The organic matter of all soils may be regarded as a substance composed of the following constituents:

1. An oxidisable complex of definite composition specific to the soil. The chief part of all soil organic matter exists in this form and accounts for 70–80 per cent. of the total carbon and nitrogen.

This fraction is mainly responsible for the varying C/N ratios obtained for different soils.

2. A non-nitrogenous complex accounting for 10–15 per cent. of the total carbon. No attempt has been made in this investigation to ascertain its nature.

3. A nitrogenous residue accounting for about 5 per cent. of the total carbon and the remainder of the total nitrogen not included in the oxidisable complex.

Although the weight of existing evidence bearing on the origin of soil organic matter tends to show that lignin is the one chief constituent of plant origin which is most resistant to the activities of micro-organisms and likely to accumulate in the soil, and that synthesised microbial substance is the other important contributor to soil organic matter and apparently accounting for its nitrogen moiety, there is no experimental evidence to prove that these two substances exist in the soil as definite components of organic matter.

Waksman, in his attempt to separate organic matter into certain broad categories, obtains in the course of the analysis a soil residue after treating the original soil with ether, alcohol, 2 per cent. HCl and 80 per cent. H_2SO_4, which he suggests contains a "lignin-humus complex" and "organic nitrogenous complexes," and then proceeds to separate them by converting its nitrogen content into protein by the conventional factor 6·25 and, after making an allowance for the carbon in the protein, he calculates the amount of a "lignin-humus complex" from the amount of carbon left over in the residue by assuming that this substance contains 62 per cent. carbon. Since the residue in question contains the bulk of the organic matter, *no useful purpose can be served by making such an arbitrary separation,* and in view of the writer's results in this investigation, an entirely erroneous conception appears to have been put forward. By stating percentages of these two complexes in an analysis, it implies that

they are present in the soil on the same footing as cellulose, for example. Moreover, it is hardly conceivable from a chemical point of view that even a closely associated colloidal complex, composed of a substance of a lignin nature and a substance of protein nature, would be oxidised by hydrogen peroxide in a progressive manner in the way that the "oxidisable complex" of the present writer is decomposed. Yet, Waksman's "lignin-humus-nitrogenous complex" and the writer's "oxidisable complex" must be for the most part labels for the same substance. Each accounts for 70–80 per cent. of the total organic matter and cellulose is excluded in each case, and it would be agreed that the other constituents of organic matter are small in comparison. Maillard's[7] demonstration of humus formation from amino acids and carbohydrates seems to offer a more reasonable explanation of the formation of a complex that would be oxidised progressively by peroxide. Maillard found that a concentrated solution of a sugar and an amino acid gave on heating a black precipitate which had the properties of soil humus. He concluded that a similar change occurred in the soil, the protein of the plant and animal residues decomposing to polypeptides and amino acids which reacted with the carbohydrates of the residue to form soil humus. It contained 4·4–6·0 per cent. nitrogen—a figure similar to that found for the percentage of nitrogen in the "oxidisable complex" of this investigation. Experiments by Beckley[8] show that humus is formed as stated by Maillard but that hydroxy-methyl-furfural is formed as an intermediate product. Again, recent work by Phillips, Weihe, and Smith[9] has shown that under suitable conditions soil organisms are capable of decomposing lignin as found in lignified plant materials, and that the rate of decomposition of lignin may be as great as that of the celluloses and pentosans. It is unlikely, therefore, that lignin or a lignin-like substance will be found as such in the organic matter of soils. Mattson[10], in a study of the various components of humus from the colloidal chemical standpoint, expresses the view that "the various components of humus reported as 'hemicellulose,' 'cellulose,' and 'lignin' may be very different from the corresponding materials present in the original plants in which they were synthesised. Oxidation and reduction, substitution and addition derivatives, and lactone formation would result in combinations possessing very different properties. It must be remembered that in the methods of analysis now in vogue, these delicately complex and reactive materials are subjected to such a drastic treatment that any conclusion as to their original make-up is extremely hazardous."

SUMMARY.

1. The attack on the organic matter of different soils using varying strengths of hydrogen peroxide has been investigated.

2. Starting with the most dilute peroxide, a complex of constant composition is oxidised in increasing amount.

3. Above a certain strength of peroxide there is no further attack on the nitrogenous compounds of the organic matter, but a progressive decomposition of non-nitrogenous carbon compounds or complexes.

4. The fraction oxidised up to the point at which no further decomposition of nitrogenous matter occurs is termed the "oxidisable complex", and, whilst apparently specific for a given soil, varies somewhat in composition in different soils.

5. The "oxidisable complex" accounts for 70–80 per cent. of the total carbon and nitrogen of soil organic matter, and is more readily decomposed in carbonate-free than in carbonate soils.

6. The C/N ratio of the "oxidisable complex" principally determines the C/N ratio of the soil.

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THE ASSIMILATION AND TRANSLOCATION OF PLANT NUTRIENTS IN WHEAT DURING GROWTH.

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(With Three Graphs and One Diagram.)

CONTENTS.

INTRODUCTION.

THE investigation described extended over two years. Since the work in the latter year was considerably more detailed, and based upon more than double the number of samples which, also, were about six times as large as those of the first year, the first year's results are discussed in their appropriate place with those obtained in the second year. This appears desirable, as obviously the first year's results are best considered

in the light of their confirmation, or otherwise, of the more detailed ones of the second and more normal season (1930). Accordingly, the data from the first year's work are given in the Appendix to avoid over-burdening the text.

In outline, the present account relates to the weight and composition of the dry matter of the wheat plant at nine different stages of growth. Each sample consisted of 3200 tillers drawn from an area of 2 acres, and as it was considered advantageous to ascertain the degree of uniformity of growth and composition over the whole area, this was laid out as an 8 × 8 Latin square of 64 plots.

Letters were allotted to the plots as shown in the diagram of the square given in the Appendix. Fifty tillers were drawn at random from each plot at every sampling, and the tillers from the plots of each letter series bulked. These samples of 400 tillers were weighed separately until harvest and analysed separately until three weeks before harvest, by which time it was considered that sufficient information regarding uniformity had been obtained.

The results revealed that the crop was extremely uniform throughout the area, both as regards weight and composition of dry matter. This may be inferred by a study of the figures given in the Appendix showing the weight of dry matter obtained from each letter series of plots at the various sampling dates. The figures showing the composition of the plants from each series are also given for the purpose of providing means of testing their reliability.

No similar investigation on an equally extensive scale is known to us, although allusion is made to some German work carried out in 1903. In this country Brenchley and Hall[1], and later Woodman and Engledow[2], have studied changes during the development of the wheat grain, and a study of the changes in the composition of the growing oat plant has been made by Berry[3].

NATURE OF SOIL, SEED AND WEATHER CONDITIONS UNDER WHICH THE CROP WAS GROWN.

The wheat was grown at Good Easter, Essex, upon land which had grown oats in 1927, and which was fallow cultivated in 1928–9. For the 1930 crop the land received an autumn application of 1 cwt. of sulphate of ammonia and 3 cwt. of superphosphate per acre, followed by 1 cwt. of nitrate of soda in the spring. The soil is heavy calcareous clay, an analysis of which is appended. The plots were on level ground, about 250 ft. above sea level and unshaded by trees. The variety "Victor"

was drilled, with a spacing between the rows of wheat of $7\frac{1}{2}$ inches, on a good seed bed on October 30, 1929, under excellent weather conditions.

Meteorological data during the growth of the plant were recorded, and are given in the Appendix, but a short summary of the effect of the general weather conditions on the crop is given here.

The wheat brairded on November 12 and made good progress throughout December, by the end of which month the plants had obtained their third leaves. Good growth and tillering occurred in January, but thereafter, until the latter part of March, the plants received a check, and some yellowing of the outer leaves was observed.

Excellent growth was made from April to the middle of July, when very wet weather and high winds caused considerable areas to be lodged. In August a heavy but rather badly lodged crop was harvested.

METHOD OF SAMPLING AND PREPARATION OF SAMPLES.

The crop was first sampled on April 30, about seven weeks before ear emergence was general, by cutting tillers about 1 inch from the ground. Fifty such tillers were taken at random from each of the 64 plots (each 1/32 of an acre). The tillers from each series of 8 plots were tied together, labelled, and removed to the laboratory for separate analysis. Each separate analysis was thus made on 400 tillers.

This procedure was repeated three weeks afterwards, then fortnightly, until three weeks before harvest and subsequently weekly.

The data showing the analyses of 3200 tillers which, with the weights of dry matter, form the basis of this study represent the average composition, deduced from the weights of nutrients present in eight separate samples of 400 tillers, at the date of each sampling.

On arrival at the laboratory, the samples were cut up by means of scissors and spread to air dry in shallow wooden trays. In the later samples, in which the ears had formed, these were removed, the straw and ear then being treated similarly but separately. In the final samples, separation was also made of chaff and grain. When thoroughly air dried, the weight of air-dry material in each sample was recorded, and the samples finely ground, and then preserved in air-tight bottles.

METHODS OF ANALYSIS EMPLOYED.

The following determinations were made on each sample:
Dry matter. By drying to constant weight at 100° C.
Nitrogen. By Kjeldahl-Gunning method.

Phosphoric acid. By destruction of the organic matter by means of sulphuric and nitric acids, precipitation as ammonium phospho-molybdate and reprecipitation with magnesia mixture.

Calcium. By burning at a low temperature, extracting the ash with hot dilute hydrochloric acid, precipitation of the calcium as oxalate and subsequent titration with $N/10$ potassium permanganate.

Ash. By ignition in platinum dish over low argand burner.

Potash. By extracting the ash obtained as above with hot dilute hydrochloric acid, removal of all bases other than soda and potash by precipitation with barium hydroxide, ammonia, ammonium carbonate and ammonium oxalate, followed by precipitation of the potash as per-chlorate.

Chlorine. By mixing to a paste with lime, ashing at a low temperature, extracting with hot dilute nitric acid, and titration with silver nitrate by Volhard's method.

Silica. By weighing the insoluble material of the ash after extraction with hydrochloric acid.

Table I. *Average percentage of nutrients in dry matter of whole plant* (1930).

	Before ear emergence			After ear emergence					
	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling	6th sampling	7th sampling	8th sampling	9th sampling
Constituent	(30. iv.)	(21. v.)	(4. vi.)	(18. vi.)	(2. vii.)	(16. vii.)	(23. vii.)	(30. vii.)	(6. viii.)
Nitrogen	3·594	1·851	1·288	0·943	0·785	0·765	0·727	0·743	0·767
Phosphoric acid	0·967	0·810	0·666	0·590	0·463	0·432	0·423	0·433	0·440
Lime	0·936	0·604	0·490	0·377	0·305	0·258	0·213	0·209	0·213
Potash	4·098	3·204	2·717	1·885	1·187	0·865	0·755	0·698	0·631
Chlorine.	0·778	0·509	0·391	0·303	0·277	0·212	0·182	0·160	0·136
Silica	2·11	2·92	3·14	2·81	2·66	2·97	2·92	3·04	3·11
Silica-free ash	8·67	7·42	5·61	4·19	2·82	2·33	2·06	1·86	1·81

PERCENTAGES OF NUTRIENTS IN DRY MATTER.

Whole plant (Table I).

It will be observed that the percentages of nutrients in the dry matter of the whole plant all decreased from the first sampling, as the plant matured, with the exception of silica which remained practically constant throughout. There can only be two reasons for such decrease, either an actual loss of the nutrients or the assimilation of a greater proportion of something else, viz. carbon. It will be seen later, when the actual weights are considered, that, although in the case of some of the nutrients taken up from the soil there is an actual loss in the later stages, there is no loss in the earlier stages, and the cause of the depression in·

percentage is therefore the slower rate at which assimilation of these substances is taking place in comparison with carbon.

In the case of silica, where no depression in percentage is noted, it follows that its rate of assimilation must at least equal that of carbon. Of the nutrients which undergo most change in percentage, the following observations may be made:

(1) Period before ear emergence: nitrogen decreases most rapidly, lime and chlorine fairly rapidly, phosphoric acid and potash more slowly, and silica increases.

(2) Period of five weeks after ear emergence: all percentages, with the exception of silica, decrease; this is most marked in the case of potash.

(3) Period of fortnight before harvest: nitrogen and phosphoric acid are practically constant during the last three weeks, lime is constant for the last fortnight, potash and chlorine continue to decrease until actual harvest.

The results for 1929 (see Appendix) show substantial agreement with the one exception of phosphoric acid, which did not decrease so early in the season of 1929 as under the more normal conditions of 1930.

Table II. *Average percentage of nutrients in dry matter of straw* (1930).

Constituent	4th sampling	5th sampling	6th sampling	7th sampling	8th sampling	9th sampling
Nitrogen	0·831	0·544	0·370	0·268	0·258	0·259
Phosphoric acid	0·541	0·343	0·184	0·148	0·125	0·129
Lime	0·409	0·345	0·325	0·296	0·299	0·307
Potash	1·955	1·250	0·921	0·847	0·814	0·761
Chlorine	0·325	0·307	0·244	0·241	0·227	0·204
Silica	3·07	2·71	3·20	3·63	3·87	4·07
Silica-free ash	4·34	2·80	2·21	2·06	1·78	1·75

Straw (Table II).

These figures call for little comment—the chief feature to be observed, viz. the great falling off in percentage of nitrogen and phosphoric acid as the plant ages, being fairly well known. There is a comparatively large fall in percentage of potash, lime and chlorine fall to a much smaller extent, while the proportion of silica is increased.

Table III (a). *Average percentage of nutrients in dry matter of ears* (1930).

Constituent	4th sampling	5th sampling	6th sampling	7th sampling	8th sampling	9th sampling
Nitrogen	1·758	1·768	1·532	1·355	1·327	1·355
Phosphoric acid	0·941	0·952	0·912	0·800	0·807	0·821
Lime	0·150	0·143	0·129	0·102	0·099	0·103
Potash	1·346	0·934	0·755	0·628	0·559	0·481
Chlorine	0·142	0·148	0·128	0·100	0·078	0·056
Silica	0·90	2·48	2·53	1·96	2·02	1·99
Silica-free ash	3·11	2·90	2·55	2·06	1·96	1·86

Ears (Table III (*a*)).

It will be seen from Table III (*a*) that the percentages of nitrogen, phosphoric acid and lime in the ear fall from about a fortnight after ear emergence until a fortnight before harvest, when a state of constancy is reached. Potash and chlorine, and silica-free ash diminish throughout. The percentage of silica is more than doubled in the fortnight following ear emergence, and drops slightly in the final three weeks.

Taking into consideration the fact that the second sampling in 1929 corresponds, as regards stage in the life-history of the plant, with the fifth sampling in 1930, it is apparent that there was no significant difference in the results.

Table III (*b*). *Percentages of nutrients in dry matter of grain and chaff.*

	Grain		Chaff	
Constituent	8th sampling	9th sampling	8th sampling	9th sampling
Nitrogen	1·581	1·574	0·517	0·582
Phosphoric acid	0·938	0·936	0·386	0·417
Lime	0·083	0·085	0·150	0·166
Potash	0·506	0·504	0·726	0·399
Chlorine	0·052	0·050	0·165	0·080
Silica	—	—	8·50	8·98
Silica-free ash	2·11	2·10	1·48	1·02

Grain and chaff (Table III (*b*)).

Table III (*b*) indicates that the percentage of nutrients in the grain remains unaltered in the week before harvest, but the percentages in the chaff undergo considerable change during that time. Thus, nitrogen, phosphoric acid, lime and silica all tend to increase, but notable decreases are observed in the case of potash and chlorine. The extent to which silica-free ash falls can well be accounted for by the large decrease in these two components.

WEIGHTS OF NUTRIENTS IN PLANT DURING GROWTH.

Since the weights of dry matter in straw and ear were determined, the figures for percentages of nutrients in the dry matter can be converted into the weights present in the plant and parts of the plant. The figures obtained in this way are now presented, discussed, and plotted in Graphs 1, 2 and 3.

(N.B. The figures given for nitrogen-free organic matter were ascertained by deducting the weight of elementary nitrogen and ash from the weight of dry matter. The term should not be confused with the more usual "nitrogen-free extractives," which represents organic matter other than protein, oil and fibre. The object, in this case, is to follow carbon

assimilation, and not the form into which the carbon is elaborated by the plant.)

Table IV. *Weight in grams of substances in 3200 tillers of wheat (whole plant, 1930).*

Constituent	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling	6th sampling	7th sampling	8th sampling	9th sampling
Dry matter	766	2968	6185	9513	12,267	14,354	15,062	14,728	14,211
Nitrogen-free organic matter	656	2606	5564	8757	11,498	13,474	14,202	13,897	13,405
Nitrogen	27·55	54·95	79·70	89·74	96·32	109·8	109·4	109·5	109·0
Phosphoric acid	7·415	24·03	41·23	56·05	56·76	61·94	63·74	63·71	63·94
Lime	7·180	17·93	30·32	35·90	37·44	37·09	31·98	30·75	30·24
Potash	31·42	95·09	168·1	179·3	145·7	124·1	113·7	102·9	89·68
Chlorine	5·962	15·10	24·20	28·81	33·86	30·48	27·36	23·61	19·28
Silica	16·21	86·73	194·5	267·1	326·9	426·7	440·6	447·4	441·6
Silica-free ash	66·46	220·4	347·0	399·0	346·0	333·6	310·2	274·0	256·0

The above table need be only briefly considered here, as its main significance is best seen after Tables V and IX (*a*) have been considered.

It will be noted that before ear emergence, intake of *all* nutrients was proceeding, but that this ceased for potash, lime and chlorine soon after ear emergence; cessation of nitrogen, phosphoric acid and carbon assimilation followed later at intervals of from two to three weeks. During the fortnight immediately before harvest, assimilation of all nutrients had ceased.

The German workers (4) alluded to observed assimilation of phosphoric acid proceeding until three weeks before the final harvest. Our own work, and also that of Pierre in France, supports that view, and agrees in showing that assimilation of phosphoric acid ceases some time before harvest. Hall, however, found assimilation of nitrogen and phosphoric acid proceeding until a week before maturity was reached. Our two seasons' work has shown that nitrogen intake ceases about three weeks earlier. In the case of oats, Berry observed absorption of phosphoric acid, total ash, and accumulation of dry matter continuing until the plant was mature.

During the later weeks of the plants' life, there were losses in varying degrees of all constituents, with the exception of nitrogen, phosphoric acid and silica. These facts and others are more clearly seen in the following table.

Weights of nutrients at each sampling expressed as percentages of maxima reached (Table V).

It is at once apparent that the plant, in its early stages of growth, contains a high proportion of the maximum quantity of nitrogen, potash,

lime and chlorine, and a low proportion of its nitrogen-free organic matter and silica. Thus, seven weeks before the plant comes into ear, it contains 25 per cent. of its nitrogen, but only about 4 per cent. of its silica.

Table V. *Amounts of nutrients in whole plant present at dates of sampling as percentages of the maxima.*

Constituent	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling	6th sampling	7th sampling	8th sampling	9th sampling
Dry matter	5·1	19·7	41·1	63·2	81·5	95·3	100	97·8	94·4
Nitrogen-free organic matter	4·6	18·3	39·2	61·7	81·0	94·9	100	97·9	94·4
Nitrogen	25·1	50·0	72·5	81·7	87·7	100	99·6	99·6	99·2
Phosphoric acid	11·6	37·6	64·5	87·7	88·8	96·9	99·7	99·6	100
Lime	19·2	47·9	81·0	95·9	100	99·0	85·4	82·1	80·8
Potash	17·5	53·0	93·8	100	81·2	69·2	63·4	57·4	50·0
Chlorine	17·6	44·6	71·5	85·1	100	90·0	80·8	69·7	56·9
Silica	3·6	19·4	43·5	59·7	73·1	95·4	98·5	100	98·7
Silica-free ash	16·7	55·2	87·0	100	86·7	83·6	77·8	68·7	64·1

Large losses of certain constituents evidently occurred during the time which followed attainment of maximum quantities, notably in potash, lime and chlorine, and silica-free ash, the amounts being no more, or even less, at final harvest than before ear emergence, about ten weeks earlier. Loss of the same constituents was observed in the previous season, the percentage losses for the two seasons being:

	1929	1930
Potash	25	50
Lime	43	19
Chlorine	32	43
Silica-free ash	20	36

Although every care was taken, the losses may be partly due to loss of leaf through falling, or rubbing off, at the final samplings, but this could by no means account for more than a small fraction of the losses. Further, the losses began at a time when the plant was still green and assimilating, and there would, therefore, be little danger of leaves breaking off. In our opinion, also, leaching by rain from an actively growing plant is extremely improbable, although we are well aware that by immersing leaves plucked from growing plants in water mineral substances can be dissolved out.

The maximum mechanical loss which could have occurred is seen by assuming that the loss of dry matter was due entirely to leaf which is relatively rich in these constituents. This could only account for about 6 to 7 grams of potash, whereas the actual loss was 12 to 15 times as high as could be accounted for, even on such an assumption.

This large loss of potash was also observed by the German workers,

who found that only 59 per cent. of the quantity which had entered the plant was present at the final harvest. The loss observed by us in 1930 was 50 per cent. compared with the 41 per cent. recorded by them. After pointing out that the loss of potash could not be due to mechanical

GRAPH I – WEIGHTS OF NUTRIENTS IN 3200 TILLERS OF WHEAT: WHOLE PLANT (1930)
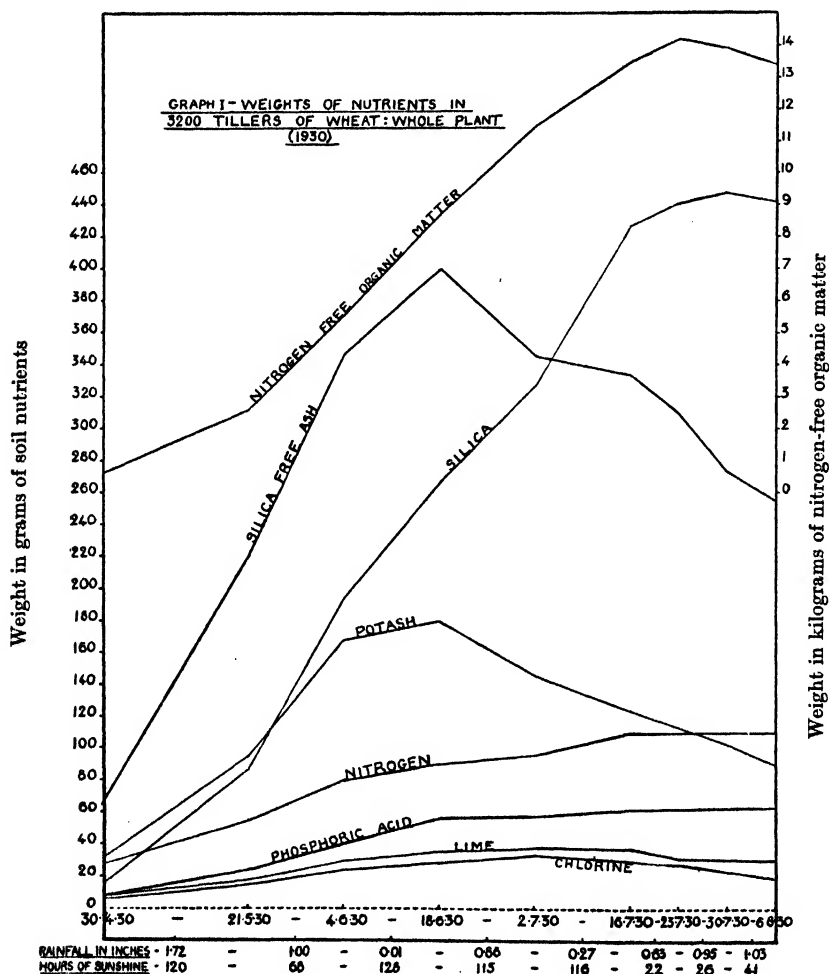
loss, since this would have had to be as high as 40 cwt. of dry matter per acre in order to account for it, the German workers observe that "the quantity of phosphoric acid at the final harvest remained practically the same as at the earlier harvests. If large quantities of the crop have been lost, why has not the phosphoric acid, at the final harvest, decreased

in the same proportion as the other constituents?" They conclude "that a part of the potash, which is assimilated, is not deposited in the form of insoluble organic substance, but in movable form, assisting in the ✔

GRAPH 2 – WEIGHTS OF NUTRIENTS IN STRAW OF 3,200 TILLERS OF WHEAT (1930)
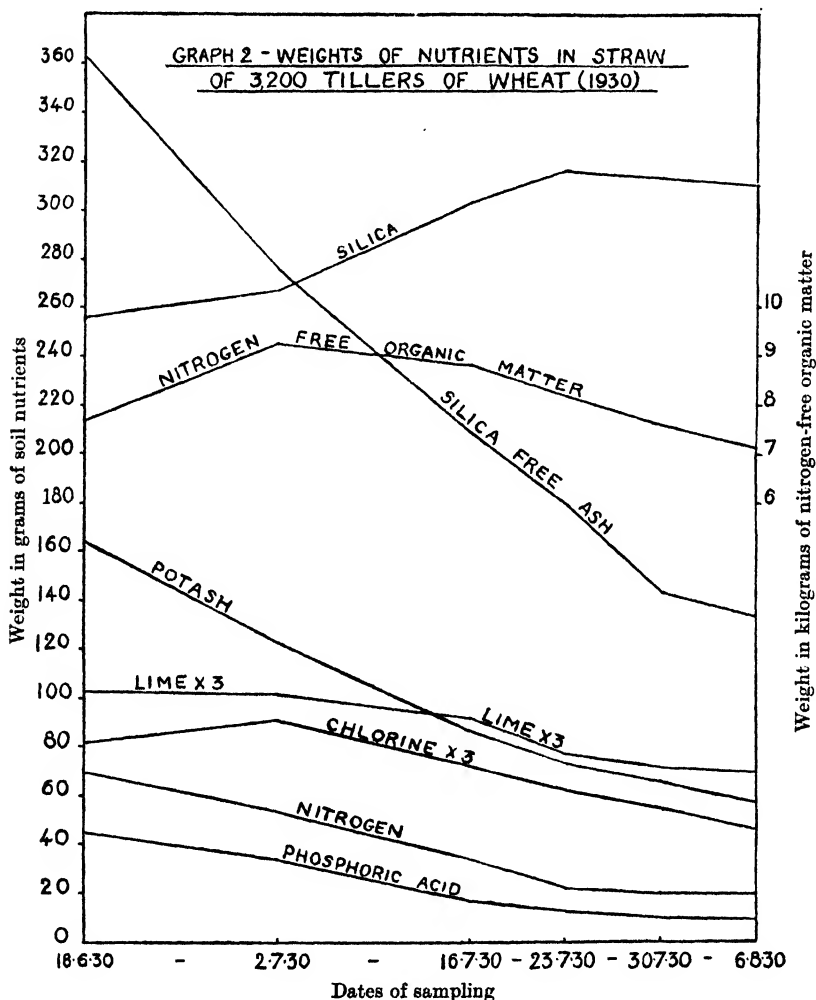
carrying out of the physiological processes within the plant, such as in the transportation of starch, etc., and when the plant approaches decay, returns again to the soil through the roots."

We could offer the same argument regarding proof that no great mechanical loss took place by pointing to the constancy of weight of

not only phosphoric acid but also of nitrogen and silica. The results of the German workers, both as regards constancy of weight of phosphoric acid and loss of weight of potash, are in agreement with our own, except as to the extent of the latter. They observed the same state of affairs
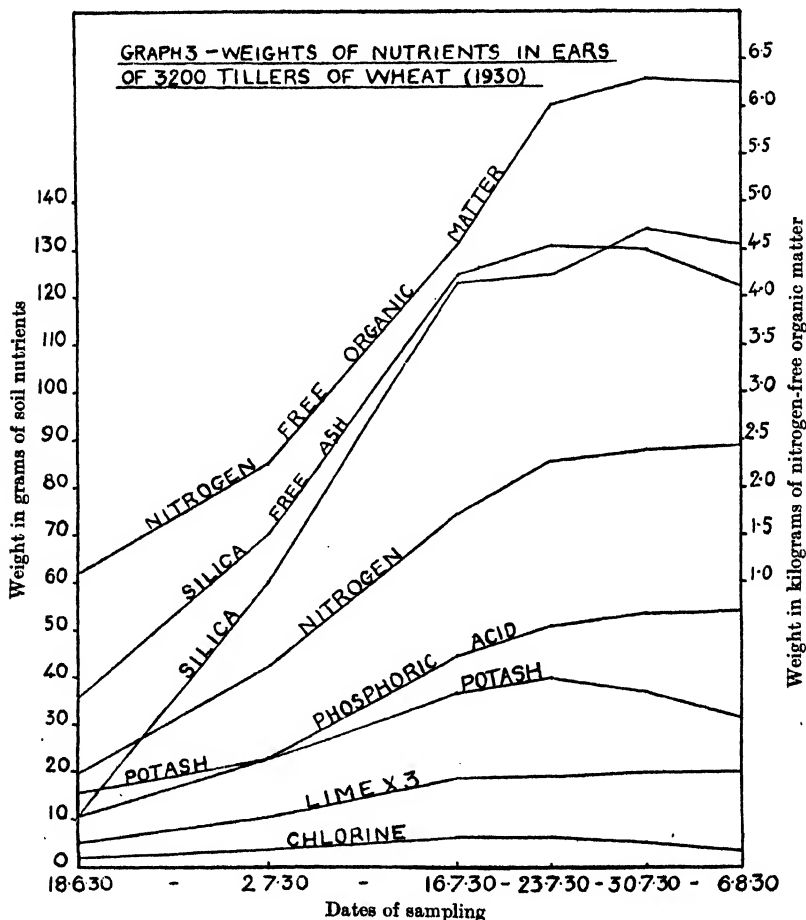
in the case of barley, and it should be pointed out here that a loss of potash was noted by Berry in the case of oats.

With regard to nitrogen, they observed a large loss. As indicated above, our results over two seasons showed that no such loss occurred.

. As lime, chlorine and silica-free ash were not determined by the

German workers, we are unable to make comparisons of the losses of these constituents.

We are aware that it has frequently and intermittently been argued that a return of nutrients to the roots occurred as plants approached maturity, and in the case of wheat we can only conclude that this takes place in regard to potash, lime and chlorine, but not in regard to phosphoric acid, nitrogen and silica.

Additional evidence of a downward movement is adduced by comparing the composition of straw and stubble (Table VI). The "stubble" in this case was obtained at the final sampling by cutting off the bottom 4 inches from the tillers, which had been cut 1 inch from the ground. The higher percentages of potash and chlorine in the stubble certainly suggest a downward movement of these two constituents.

Table VI. *Percentage of nutrients in dry matter of straw at harvest.*

	Nitrogen	Phosphoric acid	Lime	Potash	Chlorine	Silica	Silica-free ash
Straw	0·267	0·139	0·326	0·675	0·177	4·10	1·62
Stubble	0·225	0·090	0·233	1·116	0·315	4·04	2·17

The possibility of the higher percentages of potash and chlorine in the stubble being due to adhering soil can be ruled out by the method of sampling mentioned, a fact borne out by the percentage of silica, which is the same in both straw and stubble.

Consideration of the weights of nutrients in grain and chaff, as shown in Table VIII (*b*), will clearly indicate that a downward movement of potash and chlorine was taking place in the week before harvest, since about 50 per cent. of these were lost by the chaff without any increase occurring in the grain.

It may be objected that the above figures do not indicate a downward movement in the case of lime. This, however, can be explained by the fact that the main loss of lime from the straw occurred about three weeks earlier, and there was practically no loss during the last fortnight, *i.e.* there was no movement.

Certain very important consequences arise if it is the correct view that these substances are returned to the roots.

(1) It is obvious that observation of yield, coupled with analysis of the final plant, cannot be expected to give any guidance as to the manurial requirements of a crop, if only about three-quarters or one-half of the nutrients necessary for its growth are represented at that period.

(2) If substances present in such relatively large proportions as potash, lime, etc., can diminish in amount to such an extent, it is possible that substances whose presence in the plant are usually regarded as incidental or non-essential for growth may nevertheless have been present earlier in larger quantity. Moreover, substances may have been present in minute amount in the plant, may have played a rôle in its nutrition, and then returned to the root leaving no trace in the mature plant.

Table VII. *Weight in grams of substances in straw from* 3200 *tillers of wheat* (1930).

Constituent	4th sampling	5th sampling	6th sampling	7th sampling	8th sampling	9th sampling
Dry matter	8357	9849	9473	8702	8083	7626
Nitrogen-free organic matter	7668	9253	8826	8184	7605	7168
Nitrogen	69·41	53·58	35·07	23·30	20·81	19·74
Phosphoric acid	45·17	33·75	17·43	12·88	10·10	9·87
Lime	34·16	33·98	30·79	25·76	24·17	23·45
Potash	163·4	123·1	87·25	73·70	65·80	58·01
Chlorine	27·16	30·28	24·22	21·02	18·39	15·57
Silica	256·7	266·9	303·1	315·9	313·1	310·5
Silica-free ash	363·0	275·9	208·9	179·2	144·0	133·5

Total nutrients in straw (Table VII).

This table shows the extent to which the straw becomes progressively poorer in plant nutrients, and its feeding value lessened. In particular, the stocks of nitrogen, phosphoric acid and silica-free ash are being steadily depleted, whereas those of lime and chlorine are fairly well maintained until the last three weeks before harvest. Considerable losses of potash occurred from soon after ear emergence until harvest. The only marked difference observed in the two seasons was that the loss did not set in so early in 1929. In the case of silica, the stock is kept up until the end.

Table VIII (a). *Weight in grams of substances in* 3200 *ears of wheat* (1930).

Constituent	4th sampling	5th sampling	6th sampling	7th sampling	8th sampling	9th sampling
Dry matter	1156	2418	4881	6360	6645	6585
Nitrogen-free organic matter	1090	2245	4558	6018	6293	6242
Nitrogen	20·33	42·74	74·76	86·15	88·20	89·22
Phosphoric acid	10·94	23·01	44·51	50·86	53·61	54·07
Lime	1·736	3·457	6·296	6·47	6·58	6·79
Potash	15·93	22·58	36·85	39·95	37·11	31·67
Chlorine	1·646	3·581	6·261	6·341	5·221	3·707
Silica	10·38	60·05	123·6	124·7	134·3	131·1
Silica-free ash	36·06	70·05	124·7	131·0	130·0	122·5

Total nutrients in ears (Table VIII (*a*)).

These figures are probably most significant when recalculated as in Table IX, for then the rates at which the nutrients are received by the ear are easily seen.

The losses of potash and chlorine from the chaff in the two weeks before harvest have been alluded to, and are reflected in losses of these from the ear. These were not noted in the previous year.

The figures given in Table VIII (*b*) show the extent of the losses, and also that no material was being transferred to the grain during the final week.

Table VIII (*b*). *Weights in grams of substances in grain and chaff* (1930).

	Grain		Chaff	
Constituent	8th sampling	9th sampling	8th sampling	9th sampling
Dry matter	5065	5125	1580	1460
Nitrogen-free organic matter	4878	4937	1414	1306
Nitrogen	80·03	80·71	8·18	8·49
Phosphoric acid	47·51	47·98	6·10	6·09
Lime	4·21	4·36	2·37	2·43
Potash	25·64	25·84	11·47	5·83
Chlorine	2·62	2·54	2·60	1·16
Silica	—	—	134·3	131·1
Silica-free ash	106·6	107·6	23·4	14·9

RATES OF INGRESS OR EGRESS OF NUTRIENTS
IN WHEAT DURING GROWTH.

The relative rates at which the nutrients entered or left the entire above-ground plant and entered the ear, and also the rate of the nett gain and loss suffered by the straw, can be seen by calculating figures showing the percentage increase or decrease at stated samplings of the amounts present at the preceding sampling, according to whether increase or decrease occurred. These figures are given in Table IX.

When considering the straw, however, it must be remembered that, until three weeks before the final harvest, assimilation in the whole plant was proceeding, although, as far as lime, potash and chlorine were concerned, it had ceased two to four weeks earlier. Thus, in spite of the fact that the "straw had ceased to grow" three weeks earlier, it was manifestly still being used as the channel through which assimilated material was being transported in one direction or another. It should be clear, therefore, that the figures obtained for the straw in the manner mentioned indicate only the rate of the nett gain or loss, *i.e.* the balance of gains over losses or *vice versa*. In the last three weeks it is obvious that the

rate of nett loss is also the rate of gross loss, since at that time no material was entering the plant.

Before this period, however, *i.e.* when assimilation and translocation were proceeding, the straw was transporting material at a greater rate than shown by the tables. In other words, the straw undergoes a rate of "gross" loss through surrendering to the ear more material than it is itself receiving during that time.

Table IX (*a*). *Whole plant above ground. Data showing rate of nett loss or gain. Average weekly percentage increase or decrease of the amounts present at the preceding sampling.*

Sampling ...	1st–2nd	2nd–3rd	3rd–4th	4th–5th	5th–6th	6th–7th	7th–8th	8th–9th
Interval in weeks	3	2	2	2	2	1	1	1
Nitrogen-free organic matter	99	57	28	17 ·	9	5	− 2	− 4
Nitrogen	33	22	6	4	7	0	0	0
Phosphoric acid	79	36	18	1	5	3	0	0
Lime	50	34	9	2	− 1	− 14	− 2	− 2
Potash	68	38	4	− 10	− 8	− 8	− 9	− 13
Chlorine	51	30	10	9	− 5	− 12	− 14	− 18
Silica	145	62	18	11	15	3	1	− 1
Silica-free ash	77	28	8	− 6	− 2	− 7	− 12	− 7

Rates of loss or gain of nutrients by whole plant (Table IX (*a*)).

The following are the chief facts shown by these tables.

The outstanding feature is seen to be the great rate at which all nutrients are assimilated in the period seven to four weeks before ear emergence. It is evident that silica, in particular, is entering the plant very rapidly, and nitrogen comparatively slowly. From four weeks to two weeks before ear emergence there is considerable slackening off in pace, but the nutrients occupy the same positions as far as relative rates of assimilation are concerned.

Immediately before ear emergence, carbon assimilation predominates, assimilation of phosphoric acid and silica are maintained at fairly high rates, whereas that of nitrogen has much slowed down, and that of potash practically ceased. In the month following ear emergence, silica and carbon are still being taken in by the plant at fairly high rates, nitrogen and phosphoric acid are still being very slowly assimilated and ingress of lime has ceased. In the case of potash, not only has assimilation ceased, but a high rate of loss has begun; chlorine acts in a similar manner a little later in the period.

In the final three weeks before harvest, assimilation has entirely ceased with the possible exception of a small amount of carbon. Throughout this period, potash and chlorine are lost very rapidly; in the case of

lime this also applies, but only in the first week, thereafter practically no loss occurs. As mentioned earlier, there is no loss of the other nutrients.

Table IX (b). *Ears. Data showing rate of nett loss or gain. Average weekly percentage increase or decrease of the amounts present at the preceding sampling.*

Sampling ...	4th–5th	5th–6th	6th–7th	7th–8th	8th–9th
Interval in weeks	2	2	1	1	1
Nitrogen-free organic matter	53	51	32	4	– 1
Nitrogen	55	37	15	2	1
Phosphoric acid	55	47	14	5	1
Lime	50	41	3	2	3
Potash	21	31	8	– 7	– 15
Chlorine	59	37	1	– 18	– 29
Silica	239	53	1	7	– 2
Silica-free ash	47	39	5	– 1	– 6

Table IX (c). *Straw. Average weekly percentage increase or decrease of the amounts present at the preceding sampling.*

Sampling ...	4th–5th	5th–6th	6th–7th	7th–8th	8th–9th
Interval in weeks	2	2	1	1	1
Nitrogen-free organic matter	10	– 2	– 7	– 7	– 6
Nitrogen	– 11	– 17	– 33	– 11	– 5
Phosphoric acid	– 13	– 22	– 33	– 22	– 2
Lime	0	– 5	– 16	– 6	– 3
Potash	– 12	– 15	– 16	– 11	– 12
Chlorine	6	– 10	– 13	– 13	– 15
Silica	2	7	2	– 1	– 1
Silica-free ash	– 17	– 17	– 14	– 20	– 7

Rates of loss or gain by ear (Table IX (b)).

During the period seven weeks to five weeks before harvest, *i.e.* two weeks following ear emergence, the outstanding feature is the extremely rapid transference to the ear of silica, the relative rate at which migration of this is taking place being at least four times higher than that of any other nutrient. With the exception of potash, which is more slowly transferred, the remaining nutrients enter the ear at very similar speeds.

During the next two weeks the rate of migration of silica is in marked contrast to that noted in the preceding two weeks, the speed at which this is now entering the ear more nearly resembling that of the other nutrients. There is no marked falling off in the rate of the other nutrients, except in the case of nitrogen and chlorine, whereas that of the potash has increased. A week later, *i.e.* from three to two weeks before harvest, the highest rate of migration is that of carbon; nitrogen and phosphoric acid are travelling at the same speed, which is about half that of the carbon. The remaining nutrients are entering the ear extremely slowly,

if indeed at all. From a fortnight to a week before harvest assimilation of all nutrients has practically ceased, and a fairly high rate of loss of potash and chlorine begins. In the last week, the rate at which these losses are occurring is practically doubled.

In all stages of growth at which the wheat was sampled, the rate of transference of carbonaceous material to the ear has usually been higher than that of nitrogenous matter. Our observations, therefore, agree with those of Hall, in that they do not support statements that the nitrogenous matter enters the ear first, and that filling in with starch follows.

Rate of loss or gain by straw (Table IX (*c*)).

The chief features to be noted here are that losses of nitrogen, phosphoric acid and potash were occurring very rapidly during the whole period from ear emergence until harvest. Losses of lime, chlorine and carbon are also noted, but they begin about a fortnight later than in the case of the former nutrients; carbon, however, is lost comparatively slowly. Silica is assimilated at about the same rate as it is transferred to the ear.

COMPOSITION OF DRY MATTER ENTERING EAR.

From the figures given in Table VIII (*a*), showing the weights of nutrients in the ears, the composition of the material entering the ears between the sampling dates can be deduced.

The results are set out in the following table:

Table X. *Percentage composition of dry matter entering ear during successive intervals of two weeks, from ear emergence until harvest.*

Constituent	1st 2 weeks	2nd 2 weeks	3rd 2 weeks	Week before harvest
Nitrogen-free organic matter	92·8	93·9	98·3	—
Nitrogen	1·77	1·30	0·76	—
Phosphoric acid	0·95	0·87	0·52	—
Lime	0·14	0·12	0·02	—
Potash	0·93	0·58	—	—
Chlorine	0·15	0·11	—	—
Silica	2·48	2·58	0·62	—
Silica-free ash	2·90	2·22	—	—

This table emphasises certain points. It can first be noted that the material entering the ear becomes richer in nitrogen-free organic matter, poorer in nitrogen and in ash constituents.

During the last three weeks before harvest, the much diminished percentage of ash constituents in the dry matter received by the ear is made up almost entirely of phosphoric acid and silica. The falling off

in percentage of nitrogen in the dry matter entering the ear as the plant matures does not support the statement of Hall, that the material entering the grain becomes richer in nitrogen, even when allowance is made for the fact that the ear contains chaff and ear stalk which are giving up their stores of this element to the grain.

As Hall points out, however, figures showing the composition of the material entering the ear can only be viewed very generally, because the experimental errors are accumulated in quantities that are not themselves large.

DISTRIBUTION OF NUTRIENTS BETWEEN STRAW AND EAR OF WHEAT DURING GROWTH.

Table XI. *Percentage distribution between straw and ear.*

Constituent	4th sampling Straw	Ears	5th sampling Straw	Ears	6th sampling Straw	Ears	7th sampling Straw	Ears
Dry matter	87·9	12·1	80·3	19·7	66·0	34·0	57·8	42·2
Nitrogen-free organic matter	87·6	12·3	80·4	19·6	65·5	34·5	57·6	42·4
Nitrogen	77·4	22·6	70·1	29·9	32·0	68·0	21·3	78·7
Phosphoric acid	80·6	19·4	59·5	41·5	28·1	71·9	20·3	79·7
Lime	95·3	4·7	90·9	9·1	83·0	17·0	80·6	19·4
Potash	91·1	8·9	84·2	15·8	70·4	29·6	63·8	36·2
Chlorine	90·2	8·8	89·4	10·6	79·3	20·7	76·6	23·4
Silica	96·2	3·8	81·7	18·3	71·0	29·0	71·7	28·3
Silica-free ash	91·0	9·0	79·8	20·2	62·6	37·4	57·7	42·3

Constituent	8th sampling Straw	Ears	Grain	Chaff	9th sampling Straw	Ears	Grain	Chaff
Dry matter	54·9	45·1	34·4	10·7	53·7	46·3	36·1	10·2
Nitrogen-free organic matter	54·7	45·3	35·1	10·2	53·5	46·5	36·8	9·7
Nitrogen	19·0	81·0	73·1	7·9	18·2	81·8	74·1	7·7
Phosphoric acid	15·9	84·1	74·6	9·5	15·4	84·6	75·0	9·6
Lime	78·6	21·4	13·7	7·7	77·8	22·2	14·4	7·8
Potash	63·9	36·1	24·4	11·7	64·7	35·3	28·8	6·5
Chlorine	78·0	22·0	11·1	10·9	80·8	19·2	13·2	6·0
Silica	70·0	30·0	—	30·0	70·4	29·6	—	29·6
Silica-free ash	52·5	47·5	38·9	8·6	52·3	47·7	42·0	5·7

It will be apparent from the proportion of grain to straw, namely 67 parts of the former to 100 parts of the latter, that the season was not markedly abnormal. For example, the average figures given by Hall, for wheat grown at Rothamsted on unmanured land, is 70 parts of grain per 100 parts of straw; in a very wet year he observed 43 parts of grain, and in a very dry year 110 parts of grain per 100 parts of straw.

It should be noted here that in our investigations of wheat grown in the abnormally dry season of the previous year (1929), we observed 120 parts of grain per 100 parts of straw, and it is obvious that season

STRAW - ■ : EAR - [GRAIN] [STRAW]
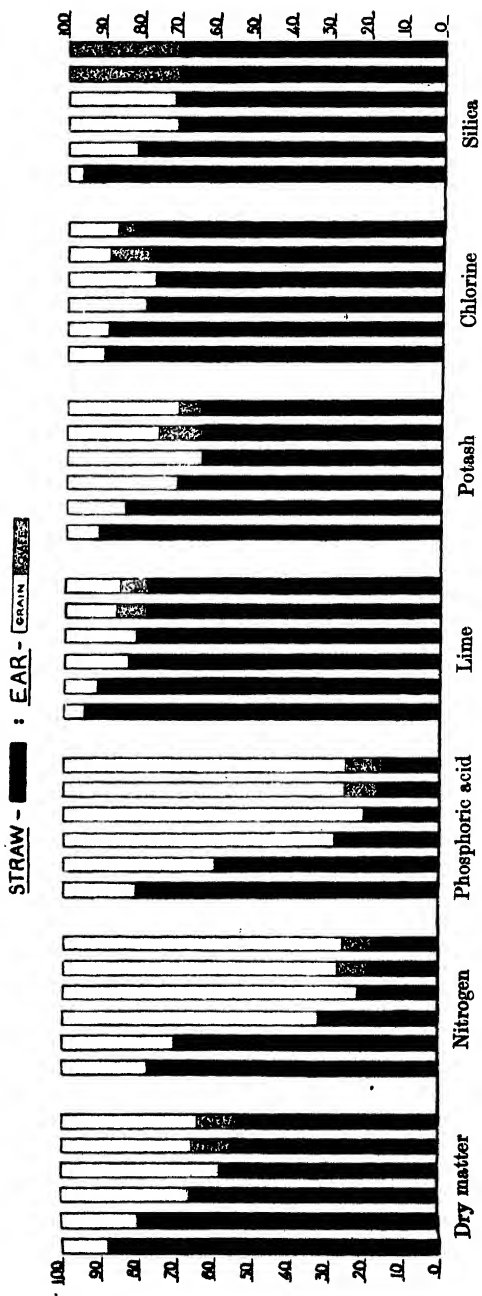
Diagram 1. Percentage distribution of nutrients in straw and ear during growth.

will have a marked influence upon the percentage distribution between different parts of the plant of substances assimilated by it.

This is well seen by contrasting the above table with the table in the Appendix, which shows the 1929 results, and indicates the extent to which the proportions of substances in the ear are increased in a very dry season.

With regard to potash, lime and chlorine, the later sampling figures for these exaggerate the extent of the migration to the ear, since large losses of these constituents from the straw occurred during the final six weeks.

It is perhaps not often realised that the chaff contains as much as 10 per cent. of the dry matter of the plant above ground, and that it may contain one-third to one-half of the plant's silica, and one-quarter of its ash.

The data are also shown in Diagram 1, which gives a clear picture of the extent and variation in the migration of the various substances throughout the plant.

SUMMARY.

1. An account has been given of the composition and weights of nutrients in 3200 wheat plants from seven weeks before ear emergence until the crop was harvested. The distribution of the plant nutrients, and the rate of assimilation and translocation of these nutrients, have been discussed.

2. In the case of the whole plant, the percentage of nutrients in the dry matter decreased from the time of the first sampling, with the exception of silica, which had a tendency to remain constant throughout. In the case of the ear, diminution in the percentage of nutrients in the dry matter was observed from a fortnight after ear emergence until harvest.

3. Marked increases in the weights of all nutrients in the whole plant were noted until ear emergence, from then onwards assimilation was much slower. The plant attained its maximum quantities of the nutrients in the following order: potash seven weeks, lime and chlorine five weeks, nitrogen three weeks, carbon, phosphoric acid and silica two weeks, before harvest.

Assimilation of these had, therefore, ceased at the above stated times, although transference to the ear proceeded until within about a week before harvest.

4. During the final six weeks, marked losses, notably of lime, chlorine

and potash occurred, which could not have been caused mechanically, or by leaching. Such losses have been recorded by other observers, but do not appear to have received full recognition. The bearing of this on manuring problems has been discussed.

5. The opinion is expressed that there is a downward movement of potash, lime and chlorine, as the plant approaches maturity. Evidence in support of this view is produced.

6. The relative rates at which nutrients are assimilated and moved in the wheat plant are shown and discussed for the first time, as far as we are aware.

7. The composition of the dry matter entering the ear is shown to become richer in nitrogen-free organic matter, and poorer in nutrients obtained from the soil as the plant matures.

8. The effect of the weather conditions on the distribution of nutrients between straw, ear and grain has been referred to.

9. The foregoing account has, in the text, applied mainly to the crop of the normal season of 1930, for reasons already given. The data, however, for the previous abnormally dry season of 1929 are given in the Appendix, from which substantially the same conclusions can be drawn.

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APPENDIX.

PLAN OF LATIN SQUARE ARRANGEMENT.

F	A	C	E	D	H	B	G
C	E	G	D	B	F	H	A
E	F	B	H	A	C	G	D
D	G	E	B	F	A	C	H
A	B	H	F	G	E	D	C
B	H	A	G	C	D	F	E
G	C	D	A	H	B	E	F
H	D	F	C	E	G	A	B

WEIGHTS IN GRAMS OF THE DRY MATTER OF 400 TILLERS OF WHEAT
FROM EACH PLOT SERIES (1930).

Plot series	1st sampling Whole plant	2nd sampling Whole plant	3rd sampling Whole plant	4th sampling		5th sampling		6th sampling	
				Straw	Ears	Straw	Ears	Straw	Ears
A	98·0	385	742	1039	144	1246	311	1160	593
B	95·3	370	786	1047	148	1238	296	1169	609
C	96·7	370	727	1037	143	1241	292	1174	598
D	93·9	368	816	1058	145	1230	293	1183	592
E	95·1	369	830	1041	145	1215	294	1191	619
F	97·1	363	730	1049	144	1248	302	1192	605
G	94·8	377	811	1028	145	1219	318	1212	614
H	95·8	366	743	1058	142	1212	312	1192	651

DATA SHOWING THE COMPOSITION OF THE DRY MATTER OF WHEAT FROM
EACH SERIES OF A TWO-ACRE SQUARE AT THE VARIOUS SAMPLING
DATES, 1930.

Plot series	1st sampling Whole plant	2nd sampling Whole plant	3rd sampling Whole plant	4th sampling		5th sampling		6th sampling	
				Straw	Ears	Straw	Ears	Straw	Ears
				Per cent. nitrogen					
A	3·586	1·862	1·294	0·833	1·698	0·561	1·615	0·367	1·514
B	3·598	1·888	1·288	0·771	1·933	0·561	1·707	0·374	1·422
C	3·547	1·800	1·337	0·811	1·697	0·569	1·744	0·383	1·498
D	3·468	1·800	1·258	0·810	1·688	0·556	1·747	0·367	1·607
E	3·608	1·832	1·318	0·847	1·652	0·528	1·724	0·374	1·487
F	3·683	1·789	1·231	0·861	1·737	0·603	1·692	0·407	1·642
G	3·627	1·969	1·343	0·816	1·659	0·477	1·954	0·359	1·606
H	3·621	1·864	1·239	0·894	1·997	0·485	1·904	0·332	1·482
				Per cent. lime					
A	0·959	0·630	0·536	0·399	0·158	0·361	0·143	0·323	0·145
B	0·946	0·608	0·496	0·410	0·149	0·361	0·177	0·330	0·126
C	0·932	0·588	0·470	0·403	0·149	0·357	0·131	0·327	0·141
D	0·895	0·593	0·469	0·407	0·142	0·320	0·144	0·323	0·109
E	0·912	0·608	0·483	0·410	0·148	0·352	0·139	0·330	0·137
F	0·939	0·575	0·459	0·409	0·140	0·342	0·140	0·315	0·129
G	0·951	0·621	0·502	0·410	0·152	0·334	0·140	0·331	0·137
H	0·956	0·607	0·507	0·421	0·163	0·342	0·135	0·317	0·126
				Per cent. silica					
A	2·17	3·05	2·82	3·30	0·774	2·38	2·44	3·15	2·50
B	2·09	2·77	3·11	3·13	0·899	2·76	2·37	3·12	2·37
C	2·05	2·91	3·19	3·08	0·934	2·78	2·90	3·27	2·44
D	2·19	2·97	3·27	3·11	0·979	2·87	2·92	3·32	2·70
E	2·21	2·97	3·11	3·14	1·003	2·78	2·60	3·39	2·47
F	1·99	3·04	2·92	3·11	0·840	2·54	2·48	3·12	2·19
G	2·01	2·71	3·22	3·03	0·884	2·73	2·75	3·11	2·35
H	2·20	2·96	3·50	2·94	0·886	2·80	3·02	3·24	2·22
				Per cent. phosphoric acid					
A	0·924	0·786	0·707	0·519	0·894	0·326	0·913	0·164	0·946
B	1·001	0·799	0·697	0·483	0·976	0·347	0·942	0·145	0·842
C	0·974	0·794	0·659	0·565	0·895	0·315	0·942	0·167	0·906
D	0·975	0·834	0·662	0·527	0·934	0·305	0·987	0·204	0·980
E	0·967	0·824	0·654	0·567	0·926	0·361	0·926	0·200	0·897
F	0·962	0·833	0·664	0·587	0·948	0·331	0·955	0·211	0·938
G	0·970	0·810	0·651	0·574	0·978	0·317	0·970	0·175	0·926
H	0·965	0·798	0·654	0·555	1·018	0·344	0·983	0·202	0·866
				Per cent. potash					
A	4·377	3·464	2·713	1·914	1·388	1·254	0·936	0·936	0·610
B	4·106	3·285	2·676	1·973	1·471	1·245	0·955	0·946	0·600
C	4·106	3·371	2·740	1·959	1·311	1·269	0·888	0·908	0·741
D	4·210	3·324	2·750	1·922	1·396	1·282	0·948	1·007	0·653
E	4·032	2·833	2·766	1·963	1·295	1·145	0·974	0·888	0·578
F	4·188	3·072	2·616	1·951	1·358	1·322	0·993	0·837	0·615
G	3·975	3·301	2·735	1·939	1·351	1·245	0·942	0·969	0·631
H	3·784	2·964	2·736	2·022	1·445	1·252	0·823	0·867	0·579
				Per cent. silica-free ash					
A	8·62	7·64	6·16	4·38	3·20	2·87	2·69	2·32	2·84
B	8·68	7·72	5·59	4·32	3·03	2·76	2·65	2·19	2·70
C	8·67	7·28	5·54	4·52	3·27	2·72	2·66	2·31	2·99
D	8·59	7·34	5·56	4·28	2·95	2·59	2·90	2·16	2·97
E	8·35	6·95	5·73	4·09	3·15	2·71	2·68	2·21	2·51
F	8·96	7·14	5·36	4·26	2·93	2·85	2·56	2·27	3·10
G	8·76	7·61	5·54	4·37	3·30	2·61	2·73	2·46	2·76
H	8·71	7·70	5·40	4·53	3·26	2·93	2·79	2·42	2·40

DATA RELATING TO 1929 CROP. VARIETY OF WHEAT—"LITTLE JOSS."
DATES CROPS SAMPLED—17. vi. 29; 8. vii. 29; 29. vii. 29; 19. viii. 29.

Percentage of nutrients in dry matter of whole plant (1929).

	1st sampling	2nd sampling	3rd sampling	4th sampling
Nitrogen (as crude protein)	12·23	7·35	6·26	6·40
Phosphoric acid	0·65	0·73	0·49	0·49
Potash	1·831	1·078	0·928	0·713
Lime	0·661	0·423	0·389	0·227
Chlorine	0·649	0·479	0·315	0·224
Silica	1·61	1·26	1·54	1·61
Ash	5·70	4·21	3·79	3·45

Percentage of nutrients in dry matter of straw (1929).

	2nd sampling	3rd sampling	4th sampling
Nitrogen (as crude protein)	6·87	3·45	1·55
Phosphoric acid	0·700	0·272	0·083
Potash	1·092	1·070	0·939
Lime	0·473	0·543	0·361
Chlorine	0·529	0·428	0·490
Silica	1·21	1·65	2·11
Ash	4·20	4·086	4·252

Percentage of nutrients in dry matter of ears and in final grain and chaff (1929).

	2nd sampling	3rd sampling	4th sampling	Grain	Chaff
Nitrogen (as crude protein)	10·83	10·02	9·70	11·21	2·79
Phosphoric acid	0·924	0·780	0·750	0·927	0·271
Potash	1·006	0·738	0·56	0·577	0·481
Lime	0·206	0·182	0·136	0·114	0·245
Chlorine	0·204	0·162	0·129	0·124	0·152
Silica	1·52	1·40	1·24	—	7·05
Ash	4·293	3·41	2·92	1·728	8·384

Weight in grams of substances in 500 tillers of wheat (1929).

	1st sampling	2nd sampling	3rd sampling	4th sampling
Dry matter	824·5	1466·5	2195·2	2132·7
Nitrogen-free organic matter	761·4	1387·5	2089·8	2037·1
Nitrogen (as crude protein)	100·87	107·85	137·3	136·6
Phosphoric acid	5·39	10·77	10·73	10·4
Potash	15·10	15·81	20·37	15·21
Lime	5·45	6·20	8·53	4·84
Chlorine	5·35	7·02	6·91	4·79
Silica	13·27	18·47	33·87	34·25
Ash	47·00	61·79	83·36	73·69

Weight in grams of substances in straw from 500 *tillers of wheat* (1929).

	2nd sampling	3rd sampling	4th sampling
Dry matter	1238·6	1257·4	862·9
Nitrogen-free organic matter	1173·3	1119·0	824·0
Nitrogen (as crude protein)	83·20	43·34	13·41
Phosphoric acid	8·67	3·42	0·72
Potash	13·52	13·45	8·10
Lime	5·73	6·83	3·11
Chlorine	6·56	5·39	3·15
Silica	15·01	20·72	18·21
Ash	52·01	51·38	36·69

Weight in grams of substances in 500 *ears of wheat and in the grain and chaff* (1929).

	2nd sampling	3rd sampling	4th sampling	4th sampling Grain	4th sampling Chaff
Dry matter	227·9	937·8	1269·8	1042·3	227·5
Nitrogen-free organic matter	214·2	890·8	1213·0	1005·6	207·4
Nitrogen (as crude protein)	24·65	93·94	123·16	116·8	6·36
Phosphoric acid	2·11	7·32	9·52	8·91	0·62
Potash	2·29	6·92	7·11	6·02	1·10
Lime	0·47	1·71	1·75	1·17	0·56
Chlorine	0·46	1·52	1·63	1·29	0·35
Silica	3·46	13·15	16·04	—	16·04
Ash	9·78	31·98	37·09	18·02	19·07

Percentage distribution between straw and ear of substances in wheat (1929).

	2nd sampling Straw	2nd sampling Ears	3rd sampling Straw	3rd sampling Ears	4th sampling Straw	4th sampling Ears	4th sampling Grain	4th sampling Chaff
Dry matter	84·65	15·35	57·3	42·7	40·5	59·5	48·8	10·7
Nitrogen-free organic matter	84·57	15·43	57·41	42·6	40·44	59·56	50·23	9·33
Nitrogen	76·0	24·0	31·6	68·4	9·8	90·2	85·5	4·7
Phosphoric acid	80·5	19·5	31·9	68·1	6·9	93·1	86·2	6·9
Potash	85·5	14·5	66·03	33·97	53·26	46·74	39·56	7·18
Lime	92·4	7·6	80·1	19·9	64·3	35·7	24·2	11·5
Chlorine	93·46	6·54	78·0	22·0	65·76	34·24	26·93	7·31
Silica	81·48	18·52	61·18	38·82	53·18	46·82	—	46·82
Ash	84·18	15·82	61·64	38·36	49·8	50·2	24·45	25·65

METEOROLOGICAL DATA DURING PERIOD OF GROWTH 1928–9.

Month	Total rain in inches	Total sunshine in hours	Highest max. temp. °F.	Lowest min. temp. °F.	Lowest grass min. °F.	Accumulated temperature (day)	
						Above 42° F.	Below 42° F.
1928							
September	0·79	219·1	81	33	27	378	11
October	2·80	113·6	67	28	22	270	17
November	1·97	62·8	59	24	19	151	33
December	2·87	60·4	54	23	15	26	157
1929							
January	1·24	43·0	48	21	14	4	270
February	0·57	64·0	51	12	4	10	329
March	0·03	183·8	68	19	10	146	141
April	1·77	158·3	70	21	14	117	76
May	1·50	265·9	78	27	18	327	31
June	0·51	209·6	78	37	25	403	6
July	1·10	251·1	85	37	26	587	4
August	1·64	185·4	87	38	34	565	1

Total rainfall 16·8 inches.

METEOROLOGICAL DATA DURING PERIOD OF GROWTH 1929–30.

Month	Total rain in inches	Total sunshine in hours	Highest max. temp. °F.	Lowest min. temp. °F.	Lowest grass min. °F.	Above 42° F.	Below 42° F.
1929							
September	0·39	183·3	86	32	28	586	6
October	2·91	119·3	65	29	19	255	29
November	4·19	61·2	60	25	15	130	67
December	3·86	67·2	57	23	11	95	90
1930							
January	1·82	44·2	59	27	21	76	61
February	0·56	68·9	51	28	21	15	139
March	1·19	139·2	60	22	14	88	94
April	2·16	115·2	67	29	23	164	36
May	2·05	169·7	70	35	28	326	11
June	0·92	245	80	36	29	521	4
July	2·39	176·9	79	43	33	550	0
August	2·55	223·8	88	38	31	587	1

Total rainfall 25 inches.
Normal annual rainfall for Eastern Counties (1881–1915) = 24·01 inches.

ANALYSIS OF AIR-DRY SOIL ON WHICH WHEAT WAS GROWN.

Chemical analysis.

	%
Moisture	8·09
Loss on ignition	5·47
Carbonate as $CaCO_3$	2·60
Nitrogen	0·154
Available potash	0·023
,, phosphoric acid	0·028

Mechanical analysis.

Fine gravel	1·96
Coarse sand	8·71
Fine sand	16·63
Silt	20·75
Fine silt	15·83
Clay	21·3

THE FUNGICIDAL PROPERTIES OF CERTAIN SPRAY-FLUIDS, VIII.

THE FUNGICIDAL PROPERTIES OF MINERAL, TAR AND VEGETABLE OILS.

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AT the present time the various forms of sulphur and sulphur derivatives of the polysulphide class, such as lime sulphur, are the chief materials available in practice for the control of the powdery mildews (*Erysiphaceae*), although washing soda has been employed with success against American gooseberry mildew (*Sphaerotheca mors-uvae*). There are varieties of fruit, such as the sulphur-shy varieties of apple, Stirling Castle and Cox's Orange Pippin, and gooseberries of the Leveller and Sulphur varieties, to which the application of any form of sulphur for the control of powdery mildews (*Podosphaera leucotricha* and *S. mors-uvae*) results, under certain conditions, in severe injury. There is therefore a special need for a fungicide which is effective against these powdery mildews without causing injury to sensitive varieties. Further, there is the general need for new fungicidal materials, as indicated in the recommendation of the Imperial Agricultural Research Conference in 1927, viz. that "in view of the paucity of materials in use...as fungicides...an investigation of the whole chemical field should be undertaken...." The general need for a substitute for sulphur becomes especially urgent in cases where the simultaneous control of a powdery mildew and sucking insects, such as aphides, is required.

Our search for a suitable sulphur substitute has therefore been confined to materials with which nicotine or pyrethrum extract could be incorporated, and the most promising compounds appeared to be oils in which these contact insecticides could be dissolved. The oils examined at Wye during the 1930 season included certain hydrocarbon oils obtained by the distillation of mineral oil or tar and by the treatment of tar products. An examination of the fungicidal properties of some of the glyceride oils was also begun.

Details of the method of emulsification of these various products and of the preparation of the sprays are given below under the separate headings. The fungicidal properties of the sprays were tested upon the conidial stage of the hop mildew (*Sphaerotheca Humuli* (DC.) Burr.), on young plants growing in the (unheated) greenhouse. Both the stage of growth of the fungus and also of the host plant were standardised according to the method described in a former article[7], where the technique of spraying is also given.

MINERAL OILS.

The use of a mineral oil emulsion against the *Erysiphaceae* appears to have been first tried by Barker and Lees[1], who used a paraffin soft-soap emulsion against *S. mors-uvae*. They reported that while the spray appeared to kill the conidia of the fungus it did not protect the foliage from re-infection. It is possible that this absence of protective power was due to the ready volatility of the paraffin oil used. With less volatile mineral oils this disadvantage might be overcome. By careful refining it has been found possible to remove, from mineral oils of higher boiling-point, the ingredients which are responsible for foliage injury, and highly refined petroleum oil (white oil) emulsions are now becoming a standard spray material for summer use.

The fungicidal properties of these products do not appear to have been closely investigated. McWorter[11] showed that, at a dilution of 1 pint in 4 gallons, "Volck," a proprietary mineral oil emulsion guaranteed to contain 80 per cent. by weight of petroleum oils, was effective in controlling *S. pannosa*. He considered that not only did this material possess protective fungicidal properties due to the non-volatility of the oil but that there was a direct toxic action upon the mildew. On the other hand, Guba[9], from a series of trials of "Volck" for the control of *Erysiphe cichoracearum* attacking greenhouse cucumbers, concluded that this material has no significant fungicidal value against this disease.

At present the highly refined high-boiling mineral oil emulsions are largely used for the control of red spider. As nicotine derivatives and pyrethrum extract are readily soluble in these oils, it is not unlikely that their use will become widespread in horticulture.

For the spray trials, two samples of petroleum oil were used: the first, a cheap grade of liquid paraffin purchased at the local chemist's shop; the second, a high grade medicinal paraffin guaranteed to be in accordance with the B.P. 1914. In addition, three proprietary mineral

oil emulsions were employed. Upon analysis, by the methods given in (10), these products gave the following figures:

•	Content of oil per cent. by weight	Of the isolated oil		
		Sp. gr.	Unsulphonated residue per cent.	Sulphur compounds
Liquid paraffin	—	0·870	98·8	Absent
Medicinal paraffin	—	0·893	100·0	Absent
"Summer solol"	61·6	0·854	98·2	Absent
"Volck"	80·0	0·876	98·7	Absent
"Sternol insecticide"	75·3	0·853	93·5	Absent

"Sternol insecticide" was found to contain, in addition to mineral oils, phenolic compounds amounting to 1·4 per cent. by weight. The presence of these compounds in the isolated oil in part accounts for the comparative lowness of the figure for unsulphonated residue.

Liquid paraffin.

Stock emulsions were prepared by the slow addition of the oil to 40 ml. 10 per cent. soft-soap solution maintained at a temperature just below boiling-point. The mixture was stirred vigorously with an egg-whisk after each addition of oil, the process being continued until 80 gm. of the liquid paraffin had been added. While the mixture was still warm the oil rapidly separated to a continuous phase, but, when cool, violent agitation of the mixture was sufficient to give a cream-like and permanent emulsion which was diluted to give the various sprays required. A similar preparation was made in which the soft-soap solution was replaced by a 10 per cent. sodium castor-oil soap solution. The emulsion so prepared was inferior in stability to the soft-soap emulsion, and separation of the oil (the breaking of the emulsion) occurred readily on standing. A satisfactory emulsion was, however, obtained by shaking the mixture.

Exp. 28. 2 per cent. liquid paraffin and 0·05 per cent. castor-oil soap.

Six leaves sprayed; the solution proved fungicidal or not quite fungicidal—due probably to uneven wetting of the patches. Slight injury was caused to the youngest leaves, especially where they were very seriously attacked by the mildew. The patches on the control leaves at the same nodes remained powdery.

Exp. 75. 2 per cent. liquid paraffin and 0·05 per cent. soft soap.

Six leaves sprayed; the solution, although it did not always run well, proved fungicidal, but there were indications that it was near the limit. No injury was caused. The patches on the control leaves at the same nodes remained powdery.

As it was possible that the uneven action observed was due to the uneven wetting of the patches by the spray, additional soap was added.

Exp. 85. 2 per cent. liquid paraffin and 0·28 per cent. soft soap.

Seven leaves sprayed; the solution proved fungicidal for all powdery patches, although there were again indications that it was only just so. No injury was caused. The patches on the control leaves at the same nodes remained powdery.

Exp. 171. 2 per cent. liquid paraffin and 0·4 per cent. soft soap.

Ten leaves sprayed, those at the same nodes being sprayed with 2 per cent. liquid paraffin and 0·4 per cent. Agral W.B.S. (Exp. 172). Both solutions proved completely fungicidal to the same degree; ten of the leaves at the same five nodes began to turn yellow by the second day after spraying and eventually fell off; the remaining leaves showed no injury, except that on two leaves (at the same node) there was very slight scorching at the margins.

Exp. 48. 4 per cent. liquid paraffin and 0·5 per cent. soft soap.

Six leaves sprayed. The emulsion proved fungicidal, the patches being obliterated. Three of the leaves showed distinct signs of injury, with yellowing margins; one leaf died by the tenth day, while the control leaf remained green; the remaining two leaves were not appreciably injured. The emulsion may be considered fungicidal at a concentration that produces injury to the leaves.

In certain experiments which were carried out during a sudden spell of abnormally hot weather when the maximum temperature outdoors reached 71° F. (the mean for the month being 60·3° F.), an injurious action upon the leaf tissue was shown.

Exp. 124. 2 per cent. liquid paraffin and 0·4 per cent. soft soap.

Nine leaves sprayed, the control leaves at the same nodes being sprayed with the liquid paraffin at the same strength with 0·4 per cent. Agral W.B.S. (Exp. 125). In both cases severe injury was caused; all the leaves were dead or had dropped off by the seventh day.

Preliminary trials were also carried out upon the action of liquid paraffin emulsified by means of sulphonated oils. The method of emulsification used was similar to that employed by Tutin (15) for the emulsification of tar oils, but it was found that the emulsifiers, which are marketed by the British Dyestuffs Corporation under the names "Agral W.B." and "Agral A.X.", are not sufficiently soluble in the liquid paraffin. Nor was a further material "Agral W.B.S.," kindly supplied by the B.D.C. for the purposes of these trials, completely satisfactory in this respect. Mixtures of 10 parts by weight of liquid paraffin and 2 parts of the Agral product were prepared. The sulphonated oil went into solution readily on warming but separated on cooling. It could easily be remixed by shaking, except in the case of Agral W.B.S. which formed a solid deposit, to remix which it was necessary to heat the mixture. For the preparation of the spray a weighed amount of the liquid paraffin-

Agral mixture was added to a small quantity of distilled water and sufficient sodium hydroxide solution to give complete emulsification was then added from a burette. The emulsion so formed was added to the remainder of the water required to give the desired strength. On the average 1 ml. $N/10$ sodium hydroxide solution was required per gram of liquid paraffin-Agral mixture used.

Exp. 126. 2 per cent. liquid paraffin and 0·4 per cent. Agral A.X.

Eight leaves sprayed, the control leaves at the same nodes being sprayed with the liquid paraffin at the same strength with 0·4 per cent. Agral W.B. (Exp. 127). Inconclusive results were obtained; the emulsion with Agral W.B. caused marked injuries at the edge of the leaf which were absent in the leaves sprayed with Agral A.X. emulsion.

Exp. 171. 2 per cent. liquid paraffin and 0·4 per cent. soft soap.

Ten leaves sprayed, the control leaves at the same nodes being sprayed with the liquid paraffin at the same strength with 0·4 per cent. Agral W.B.S. as the emulsifier (Exp. 172). The emulsion proved fungicidal. Some of the sprayed leaves showed a yellowing two days after spraying, and slight scorching at the edges by the twelfth day.

Exp. 148. 0·4 per cent. Agral W.B.

Eight leaves sprayed. The solution showed no fungicidal power, the patches being all powdery again by the third day. Slight but distinct injury was frequently caused to the leaf in the form of minute, dark brown spots or flecks scattered over the lamina, which sometimes extended through the tissue to the lower surface.

Exp. 149. 0·4 per cent. Agral A.X.

Eight leaves sprayed. The solution was non-fungicidal, the patches being all powdery again by the third day. No injury was caused.

From these trials it may be concluded that, provided efficient wetting of the fungus patches is secured, the spray is fungicidal at a content of 2 per cent. liquid paraffin. Under the ordinary greenhouse conditions which obtained during the earlier part of May, no serious injury was caused to the leaves, but during a spell of hotter weather, this concentration proved capable of causing severe injury. It must be left to further experiments to ascertain at what strength the liquid paraffin is safe to use on hop plants in the open.

When liquid paraffin is emulsified by means of sulphonated oils, the 2 per cent. strength proved injurious to the leaves under the conditions obtaining. In two experiments carried out at the same time, the emulsifier Agral W.B. caused distinct, though slight, injury to the leaf tissue, while Agral A.X. caused no injury.

Medicinal paraffin.

The oil was emulsified with castor-oil soap and with soft soap by the method described for liquid paraffin. With both soaps a fairly stable paste-stock emulsion was produced; that with soft soap showed free oil after standing eight weeks. On dilution, however, the emulsion broke with great rapidity and it is possible that, during the process of spraying, although the spray bottle was continually shaken, an uneven application of the oil resulted through rapid creaming.

Exp. 10. 2 per cent. medicinal paraffin and 0·05 per cent. castor-oil soap.

Five leaves sprayed; the solution ran well and the patches appeared obliterated. It proved non-fungicidal, the patches being again powdery by the fifth day.

Exp. 47. 2 per cent. medicinal paraffin and 0·05 per cent. soft soap.

Six leaves sprayed. The emulsion proved non-fungicidal with perhaps some checking action.

Exp. 99. 2 per cent. medicinal paraffin and 0·5 per cent. soft soap.

Four leaves sprayed. The patches were much affected, but a few clustered or scattered conidiophores were produced by the ninth day. The emulsion was therefore not quite fungicidal. The patches on the control leaves at the same nodes remained powdery.

Exp. 22. 3 per cent. medicinal paraffin and 0·075 per cent. castor-oil soap.

Six leaves sprayed. On the third day after spraying the patches were practically obliterated, yet scattered conidiophores occurred on the original patches; these were still present on the ninth day. The emulsion was not quite fungicidal.

Exp. 76. 3 per cent. medicinal paraffin and 0·075 per cent. castor-oil soap.

Seven leaves sprayed. The emulsion ran well and the patches appeared obliterated as a whole, yet scattered or isolated conidiophores could still be detected. By the eleventh day there were small clusters of conidiophores from the youngest patches and scattered or isolated conidiophores from the older. The emulsion was almost fungicidal. The patches on the control leaves at the same nodes remained powdery.

It was evident that the fungicidal activity of the oil is definitely lower than that of liquid paraffin. At a concentration of 3 per cent. medicinal paraffin, fungicidal action was not complete.

Summer solol.

When diluted with distilled water the emulsion showed no tendency to break and, as the cream separated slowly, no difficulty was encountered in the application of this material.

Exp. 4. 2 per cent. solol.

Five leaves sprayed. The emulsion, which did not run well, proved non-fungicidal.

The emulsion did not wet completely the mildew patches and, as it has been shown in previous work (6) that to secure a satisfactory test of fungicidal action the spray must be able to displace the air from the densely packed conidiophores of the fungus, a spreader was added to the emulsion. In the trials with summer solol, soft soap was employed as the spreader.

Exp. 9. 2 per cent. solol and 0·5 per cent. soft soap.

Five leaves sprayed. By the fifth day the patches, which appeared semi-obliterated after the spraying, had re-grown conidiophores in scattered clusters. The emulsion, although non-fungicidal, had exerted some checking action.

Exp. 21. 3 per cent. solol and 0·5 per cent. soft soap.

Five leaves sprayed. The mildew patches were often at once semi-obliterated; and were killed. No injury was produced. Indications were present, viz. in the killing of the "hump" stage of the mildew (7), that the emulsion was super-fungicidal for the powdery stage. The patches on the control leaves at the same nodes remained powdery.

Exp. 74. 2·5 per cent. solol and 0·5 per cent. soft soap.

Five leaves sprayed. The emulsion proved nearly or quite fungicidal. The patches on the control leaves at the same nodes remained powdery.

In the above trials only the upper surface of the leaf was sprayed, but in practice, of course, the whole plant would receive the spray. To test under such conditions the likelihood of spray injury, an entire stem of the hop plant was sprayed.

Exp. 134. 2·5 per cent. solol and 0·5 per cent. soft soap.

All the leaves of a growing stem (about 3 ft. high) were sprayed on both sides of the leaf. No injury was caused. The emulsion appeared to be just fungicidal for the older, powdery patches, although fresh conidiophores grew from the youngest patches in the "hump" stage.

It is evident that a concentration of the stock emulsion of 2·5 to 3 per cent. is necessary before fungicidal strength is attained. At these concentrations the amount of mineral oil present is 1·5 to 1·9 gm. per 100 ml. At these strengths no injury was caused to the sprayed leaf.

Volck.

The behaviour of this product on dilution with water was similar to that of solol. Owing to the poor spreading properties of the diluted emulsion, an unsatisfactory wetting of the fungus occurred in the first trial. In subsequent trials, Agral I was used as the spreader. It is possible that the ineffectiveness of "Volck" in Guba's trials (9) is connected with the poor spreading properties of the diluted emulsion.

Exp. 3. 2 per cent. Volck.

Four leaves sprayed. The emulsion did not run well and proved non-fungicidal. Even on the first day after spraying, the patches were white and bore conidiophores.

Exp. 8. 2 per cent. Volck and 0·5 per cent. Agral I.

Five leaves sprayed. The patches appeared almost obliterated after spraying, and the emulsion proved fungicidal for nearly all the patches, and nearly so for the remainder, on which a few, mostly isolated, conidiophores appeared.

Exp. 20. 3 per cent. Volck and 0·5 per cent. Agral I.

Four leaves sprayed. The patches appeared obliterated after spraying. The emulsion proved fungicidal, or even super-fungicidal as judged by its effect on the "hump" stage. No injury was produced to the leaves.

Exp. 71. 2·5 per cent. Volck and 0·5 per cent. Agral I.

Five leaves sprayed. The emulsion proved fungicidal. No injury was produced.

Exp. 120. 2·5 per cent. Volck and 0·5 per cent. soft soap.

Ten leaves sprayed, the leaves at the opposite nodes being sprayed with Volck at the same concentration but with 0·5 per cent. Agral I as the spreader (Exp. 121). In both experiments the emulsions proved to be just fungicidal.

In Exps. 20 and 71 it was observed that some injury to the leaf tissue had occurred where the spray had dropped upon the underlying and older leaves. An entire stem of the hop plant was therefore sprayed.

Exp. 135. 2·5 per cent. Volck and 0·5 per cent. Agral I.

All the leaves were sprayed, on both sides of the leaf, of a stem about 3 ft. high. By the eleventh day signs of injury were apparent, on most of the leaves, in the form of brown spots on the lamina, and small patches of dead cells at the edges. Under certain greenhouse conditions, therefore, Volck at this concentration causes slight but noticeable injury.

The strength of Volck necessary to give fungicidal action therefore lies between 2·5 and 3 per cent., at which concentrations the amount of mineral oil present is from 2·0 to 2·4 per cent. At these strengths, injury to the hop plant may be caused under certain conditions.

Sternol insecticide.

As in the case of Volck and solol, the addition of a spreader was necessary to secure the wetting and penetration of the mildew patches by the spray.

Exp. 15. 2 per cent. sternol.

Five leaves sprayed. Although the patches appeared semi-obliterated after spraying, the solution proved non-fungicidal, the patches being again powdery by the sixth day.

Exp. 65. 2 per cent. sternol and 0·5 per cent. soft soap.

Eight leaves sprayed. On six of the leaves all the patches were killed; on two leaves a few of the patches produced clustered conidiophores. The patches on the control leaves at the nodes remained densely powdery. The emulsion appeared to be at, or just below, fungicidal strength.

Exp. 155. 2·5 per cent. sternol and 0·5 per cent. soft soap.

Six leaves sprayed. The patches appeared obliterated after spraying; nearly all the patches were killed, but on certain portions of two leaves a few conidiophores developed from a few patches—indicative of unequal action of the spray fluid. The patches on the control unsprayed leaves at the same nodes remained densely powdery. No injury was caused to the sprayed leaves.

Sternol insecticide proved, therefore, almost fungicidal at 2·5 per cent. concentration (with 0·5 per cent. soft soap). At this concentration the content of mineral oil is 1·9 gm. per 100 ml.

Conclusions.

The examination of the fungicidal properties of spray fluids by the method used in the above experiments supplied information upon the direct fungicidal action and on the action of the spray upon the leaf tissue. It is desirable that there should be a wide margin between the minimum strength necessary for complete fungicidal action and the maximum strength which may be applied with safety to the foliage.

The experiments indicate that, with the mineral oils examined, there is not a wide margin between toxic action on the fungus and injurious action upon the foliage. With the exception of medicinal paraffin, the toxic action of the various oils used does not differ widely. There is no conclusive evidence that the ease of breaking of the emulsion has an effect upon fungicidal properties similar to that observed in the case of the toxic action of these oils upon scale insects (5). Indeed, the medicinal paraffin emulsion, which broke with the greatest ease, had an inferior fungicidal value, though this may be due to the difference in viscosity and chemical character of the oil itself.

With regard to the foliage-injuring properties of the oils, American experience (4, 17) has shown that direct injury to leaf tissue by petroleum oils is governed mainly by two factors: first, by the presence of aromatic and unsaturated hydrocarbons, freedom from which is shown by a high percentage of unsulphonated residue or by a low iodine value; second, by the presence of sulphur compounds. In the oils examined, sulphur was absent by the "doctor" test. Further, the samples (with the exception of sternol insecticide) are comparatively free from aromatic and unsaturated hydrocarbons. It would seem questionable whether by higher refining the causes of foliage injury would be removed.

It cannot, therefore, be claimed that the mineral oils of the type examined show great promise as sulphur substitutes, but their slight fungicidal properties may add to their value as solvents for more potent insecticides or fungicides.

TAR OILS.

The possibilities of the use of tar oils as fungicides are indicated by their widespread use for the preservation of timber. The nature of the toxic constituents of creosote and tar-oil fractions of higher boiling-point towards wood-destroying fungi had been the subject of controversy. Bateman (2) concluded that, of the higher boiling-point fractions, the tar acids and bases were the active fungicides, but his views have been opposed by Dehnst (3), who showed that the removal of tar acids and bases did not reduce the toxicity of the high-boiling oil towards certain of the fungi examined.

It has long been known that untreated tar oils are highly toxic to foliage, and it has been found dangerous to apply the earlier types of carbolineum wash as ovicides to trees not completely dormant. Molz (12) observed, however, that the tolerance of foliage to carbolineum washes varies greatly with different varieties of plants, and Tutin (15) showed that, by the removal of tar acids, the toxicity of the high-boiling tar oils is greatly reduced. Thus, at a concentration of 2 per cent., the high-boiling "neutral" fraction, when emulsified with castor-oil soap, caused no injury to plum foliage. Mustard seedlings sprayed with this wash, though damaged, were not killed, whereas the same concentration of tar oils from which tar acids had not been removed killed 85 per cent. of the seedlings. It was therefore considered worth while to examine the action of the "neutral" tar-oil fraction of high boiling-point on the hop powdery mildew.

For this purpose two samples of high-boiling tar oils from which the

greater part of the tar acids had been removed by the distillers were employed. The oils were emulsified with a 10 per cent. castor-oil soap solution by the methods used for liquid paraffin. Satisfactory stock emulsions were produced which were diluted to give the concentration of tar oils required for the spray. In addition, two proprietary tar-oil preparations were used; the first, a typical sample of the modified Long Ashton wash consisting of a stock emulsion of high-boiling "neutral" tar oils, the second, a "two-solution" wash stated by the makers to be prepared in accordance with the Long Ashton recommendations (14) and made by the solution of 2 parts of Agral W.B. in 10 parts of high-boiling "neutral" tar oils. To prepare the emulsion this latter material was added to the required volume of water and sufficient alkali added to give complete emulsification.

Analysis of these products (10) gave the following figures:

	Percentage by weight			
	Neutral oil	Tar bases	Tar acids	Total tar oils
"Neutral" tar oils:				
Sample I, B.P. 280–360° C.	92·7	6·5	0·1	99·3
Sample II ,, ,,	92·3	6·2	1·4	99·9
Modified L.A. wash:				
Sample III, B.P. >260° C.	60·0		2·0	62·0
Two-solution L.A. wash:				
Sample IV, B.P. >260° C.	82·0		4·8	86·8

In all trials in which these materials were applied at an oil concentration of over 0·5 per cent. the leaves were killed. At greater dilutions, the addition of a spreader was necessary to enable the spray to wet the mildew patches.

Exp. 42. 0·5 per cent. sample I and 0·5 per cent. castor-oil soap.

Five leaves sprayed. The emulsion caused fatal injury; the leaves turning brown and dying by the third day.

Exp. 43. 0·5 per cent. sample III (= 0·30 per cent. "neutral" tar oils).

Six leaves sprayed. Serious injury was produced by the seventh day, the leaves yellowing and dying.

Exp. 58. 0·4 per cent. sample III (= 0·24 per cent. "neutral" tar oils) and 0·25 per cent. soft soap.

Six leaves sprayed. Distinct injury to the leaves resulted, although less than in Exp. 43.

Exp. 44. 0·5 per cent. sample IV (= 0·41 per cent. neutral tar oils).

Six leaves sprayed. Fatal injury was caused in three days.

Exp. 131. 0·3 per cent. sample IV (= 0·25 per cent. neutral tar oils) and 0·5 per cent. soft soap.

Five leaves sprayed. No injury was caused; the emulsion had some fungicidal action.

Exp. 87. 0·12 per cent. sample IV (= 0·10 per cent. neutral tar oils) and 0·5 per cent. soft soap.

Seven leaves sprayed. No injury was caused; the emulsion was non-fungicidal, with only a slight checking action.

Following the commercial practice, the term neutral has been applied, in the above experiments, to oils still containing the tar bases and from which only the greater part of the tar acids have been removed. Because of the serious injury produced by such oils, trials were made with a tar oil from which the remainder of the tar acids and the tar bases were extracted as completely as possible by repeated washing of an ethereal solution of the oil, first with dilute alkali, and then with dilute hydrochloric acid. The solvent was then distilled off and stock emulsions of the oil prepared with castor-oil soap and with soft-soap solution.

Exp. 41. 0·5 per cent. neutral tar oils and 0·26 per cent. castor-oil soap.

Five leaves sprayed. The emulsion produced fatal injury.

Exp. 55. 0·25 per cent. neutral tar oils and 0·44 per cent. castor-oil soap.

Six leaves sprayed. Serious injury was caused to the leaves by the third day.

Exp. 86. 0·1 per cent. neutral tar oils and 0·5 per cent. soft soap.

Six leaves sprayed. Injuries were caused to the leaves in the form of brown discolorations on the under surface of the leaf; these were usually, but not always, opposite to where the mildew patches were situated.

In no case, therefore, is the spray toxic to the mildew without injury to the leaf. The occurrence of injury to foliage by tar oils freed from tar acids appears to be contradictory to Tutin's conclusion, though it should be pointed out that Molz[12] showed that liability to injury by carbolineum washes varied greatly with foliage of different species. On the other hand, the general experience in America is that, as mentioned in the preceding section, petroleum oils containing unsaturated and

aromatic hydrocarbons are liable to give foliage injury. As the neutral tar-oil fraction consists of almost 100 per cent. aromatic hydrocarbons, our results are in keeping with this generalisation.

The correlation of foliage-injuring properties and aromatic character of the hydrocarbons is further illustrated by the results of the spray trials to determine the fungicidal properties of "tetralin" and "dekalin." These two materials, produced by the hydrogenation of naphthalene, are widely used for industrial solvent purposes. They appear to be of promise for pest control purposes, especially as solvents for water-insoluble insecticidal and fungicidal substances. Thus Woodman[18] has suggested that the well-known toxic properties of naphthalene towards red spider could be utilised in spray form by means of an emulsified solution of this substance in tetralin or dekalin. Further, the substances are more easily standardised by chemical means than solvents such as hydrocarbon and glyceride oils.

For the preparation of the sprays, in which Agral I was used as the spreader, the required amount of these substances was shaken with 0·5 per cent. solution of Agral I; a satisfactory emulsion was obtained, though, in the case of tetralin, breaking of the cream occurred on standing. Stock emulsions were also prepared, 80 gm. of tetralin or dekalin being emulsified by means of 20 ml. 10 per cent. soft-soap solution. Whereas, in this case, tetralin gave a good permanent emulsion, the dekalin emulsion was very unstable.

Exp. 1. 2 per cent. dekalin and 0·5 per cent. Agral I.

Five leaves sprayed. The emulsion was non-fungicidal: no injury was caused.

Exp. 2. 2 per cent. tetralin and 0·5 per cent. Agral I.

Five leaves sprayed. The emulsion caused injury to the leaves, the tips and edges being brown and dead by the first day after spraying. Ultimately the patches were killed, together with the underlying leaf tissue.

Exp. 30. 1 per cent. tetralin and 0·28 per cent. soft soap.

Seven leaves sprayed. Most of the patches were killed by the tenth day, but a few produced scattered or clustered conidiophores. The action appeared to be somewhat unequal, varying from fungicidal to not quite fungicidal.

Exp. 84. 1·5 per cent. tetralin and 0·16 per cent. soft soap.

Six leaves sprayed. Slight injury was caused to the tips of most of the leaves; the action of the emulsion was very variable; some of the patches being killed, others were greatly checked, while some were not affected at all.

Exp. 49. 2 per cent. dekalin and 0·5 per cent. soft soap.

Five leaves sprayed. The emulsion proved non-fungicidal, although some of the patches were greatly checked.

Dekalin, at 2 per cent. concentration, is therefore non-fungicidal and non-injurious to leaf tissue. Tetralin, at this strength, is injurious to the leaf. Tetralin, which consists mainly of 1 : 2 : 3 : 4-hydronaphthalene, still retains aromatic and unsaturated properties. Dekalin consists mainly of decahydronaphthalene, which is completely saturated and has no aromatic properties. The absence of foliage injury with the latter compound is in agreement with the general hypothesis that, with hydrocarbons of high boiling-point, unsaturation gives rise to foliage-injuring properties.

The experiments with tetralin, recorded above, indicate that at a concentration not causing injury to the leaf, this substance is not sufficiently toxic to the fungus to warrant its recommendation for use against the hop powdery mildew.

VEGETABLE OILS.

Although, by virtue of their physical properties, all are called oils, the vegetable and animal oils differ widely in chemical properties from the mineral and tar oils. The latter consist mainly of hydrocarbons, whereas the whole range of vegetable and animal oils are mixtures of triglycerides, compounds produced by the combination of glycerol and fatty acids. In beginning a survey of the fungicidal properties of the natural fats and oils, a selection of the commoner vegetable oils was examined.

Representatives of the semi-drying and non-drying vegetable oils were chosen, special attention being paid to those members whose use for insecticidal purposes had already been proposed. Staniland[13] recommended a rape-oil emulsion as a contact insecticide, selecting rape oil on account of its cheapness and ease of emulsification with soft-soap solutions. Tutin[16] used rape oil for the solution of pyrethrum extract and for the preparation of a pyrethrum spray fluid in which Agral W.B. was used as the emulsifier.

Of the semi-drying oils, cottonseed and sesame oils were used. Of the non-drying oils, rape oil was selected as the representative of the seed oils of cruciferous plants which are characterised by the presence of the glyceride of erucic acid; olive oil and peach-kernel oil represented the oils containing a large proportion of oleic acid; castor oil, in which the fatty acid present is mainly ricinoleic acid, a hydroxylated fatty acid, was also used.

The samples of oil used were of the ordinary commercial grade and

were not subjected, at this early stage of the investigation, to analytical examination. The sprays were prepared either by the dilution of a stock emulsion or by the two-solution method employed with the tar and mineral oils. The stock emulsions were prepared by shaking 1 part by volume of the oil with 2·5 parts of 10 per cent. soft-soap solution. As observed by Staniland, the extent of emulsification varied greatly with the different oils, rape oil giving immediately a permanent emulsion. With peach-kernel oil and olive oil, the emulsion tended to cream, but little free oil separated on standing. With cottonseed and sesame oils the emulsions broke readily on standing, while castor oil proved most difficult to emulsify by this method.

The two-solution washes were prepared by dissolving 2 parts by weight of Agral W.B. or Agral W.B.S. in 10 parts of oil. When required for spraying, this mixture was added to the requisite volume of water and sufficient alkali added to give complete emulsification. Approximately 10 ml. $N/10$ sodium hydroxide solution was required per 6 gm. oil-Agral mixture.

Cottonseed oil.

Exp. 168.   2 per cent. oil and 0·5 per cent. soft soap.

Six leaves sprayed. The patches appeared obliterated after spraying and were killed. No injury was caused. There were indications that the emulsion was super-fungicidal for the powdery stage, as the mildew in the "hump" stage was killed.

Exp. 187.   1 per cent. oil and 0·5 per cent. soft soap.

Six leaves sprayed. The emulsion proved fungicidal, and again there was evidence of its super-fungicidal nature.

Exp. 198*.   1 per cent. oil and 0·5 per cent. soft soap.

Seven leaves sprayed; the leaves at the same nodes were sprayed with cottonseed oil at the same strength and 0·2 per cent. Agral W.B.S. (Exp. 198). The emulsions behaved the same; while fungicidal for nearly all the patches, a few patches survived and produced a few conidiophores.

Exp. 191.   0·5 per cent. oil and 0·5 per cent. soft soap.

Eight leaves sprayed. The emulsion proved fungicidal even for the "hump" stage of the fungus. The patches on the control unsprayed leaves at the same nodes remained densely powdery.

Exp. 203.   0·25 per cent. oil and 0·5 per cent. soft soap.

Five leaves sprayed. The emulsion was fungicidal for nearly all the patches; very rarely a few conidiophores were produced at the edge of the patch. The patches on the control leaves at the same nodes remained densely powdery.

Exp. 212. 1 per cent. oil and 0·5 per cent. soft soap.

In order to ascertain whether injury would be produced if the hop plant were sprayed all over, a stem some 3 ft. high and all its leaves (on both sides) and the growing point were thoroughly sprayed. No injury resulted.

Exp. 186. 1 per cent. oil and 0·2 per cent. Agral W.B.S.

Five leaves sprayed. The emulsion proved non-fungicidal, the patches were seen to be regrowing by the second day after spraying and, by the ninth day, were again powdery.

Exp. 139. 2 per cent. oil and 0·5 per cent. Agral W.B.

Six leaves sprayed. The emulsion proved fungicidal, with evidence that it was super-fungicidal for the powdery stage.

Exp. 207. 1 per cent. oil and 0·25 per cent. Agral W.B.

Eight leaves sprayed. The emulsion proved fungicidal, with a remarkable "cleansing" action; the patches appeared "obliterated" by the third day, and by the ninth day the mycelium had almost completely disintegrated.

Exp. 208. 0·25 per cent. oil and 0·25 per cent. Agral W.B.

Eight leaves sprayed. The solution was fungicidal, with a similar, though not so marked, "cleansing" action.

It may therefore be concluded that cottonseed oil, emulsified with 0·5 per cent. soft soap, is fungicidal at a strength between 0·25 and 0·5 per cent. In one anomalous case (Exp. 198*), a strength of 1 per cent. failed to kill all the patches. When Agral W.B., at 0·25 per cent. strength, is used as the emulsifier, cottonseed oil is fungicidal at 0·25 per cent. strength. A concentration of 1 per cent., with 0·5 per cent. soft soap as the emulsifier, caused no injury to the young growing hop plant.

Sesame oil.

Exp. 181. 2 per cent. oil and 0·5 per cent. soft soap.

Seven leaves sprayed. The patches were practically obliterated on spraying, and by the ninth day all the mycelial hyphae had disintegrated, including those of the "hump" stage. No injury was caused. The patches on the control leaves at the same nodes remained densely powdery.

Exp. 175. 1 per cent. oil and 0·5 per cent. soft soap.

Four leaves sprayed, those at the same node being sprayed with 1 per cent. rape oil and 0·5 per cent. soft soap (Exp. 174, see below). The sesame-oil emulsion again proved super-fungicidal (killing the "hump" stage) with the same disintegrating action; the rape-oil emulsion proved non-fungicidal, although with some checking action.

Exp. 189.   0·5 per cent. oil and 0·5 per cent. soft soap.

Six leaves sprayed. All the patches were killed, including those in the "hump" stage, although some remained whitish or only semi-obliterated. The patches on the control leaves at the same nodes remained densely powdery.

Exp. 201.   0·25 per cent. oil and 0·5 per cent. soft soap.

Nine leaves sprayed. The patches remained checked for some eight days after spraying, but by the fourteenth day the emulsion had proved to be non-fungicidal, the patches being now again powdery.

Exp. 211.   1 per cent. oil and 0·5 per cent. soft soap.

A stem some 3 ft. high and all its leaves (on both surfaces) and growing point were thoroughly sprayed; no injury was produced.

Exp. 182.   2 per cent. oil and 0·4 per cent. Agral W.B.S.

Four leaves sprayed. The emulsion proved super-fungicidal, and the patches were obliterated. No injury was caused.

Exp. 183.   1 per cent. oil and 0·2 per cent. Agral W.B.S.

Five leaves sprayed. The emulsion proved fungicidal for the powdery stage and also usually for the "hump" stage, although occasionally a "hump" was able to produce a few conidiophores.

Exp. 206.   0·5 per cent. oil and 0·4 per cent. Agral W.B.S.

Seven leaves sprayed. The emulsion proved fungicidal with a good "cleansing" action, the mycelium being shrivelled up or disintegrated. The control (unsprayed) leaves at the same nodes remained powdery.

Exp. 184.   4 per cent. oil and 0·5 per cent. Agral I.

Seven leaves sprayed. The emulsion proved fungicidal, the patches being obliterated. No injury was produced.

The above experiments permit the following conclusions: that sesame oil, with 0·5 per cent. soft soap as the emulsifier, is fungicidal at a strength between 0·5 and 0·25 per cent.; that, with 0·4 per cent. Agral W.B.S. as the emulsifier, sesame oil is fungicidal at 0·5 per cent. strength; that, at a concentration of 4 per cent., sesame oil causes no injury to the young growing hop plant.

Rape oil.

Exp. 165.   4 per cent. oil and 0·5 per cent. soft soap.

Four leaves sprayed. The emulsion proved fungicidal, the mildew in the "hump" stage also being killed. Slight injury was caused in the form of small, limited brown areas of dead leaf cells situated round the mildew spots.

Exp. 180. 3 per cent. oil and 0·5 per cent. soft soap.

Four leaves sprayed. The emulsion proved fungicidal, the patches being obliterated. Very slight "scorching" injury was caused at the edges and tips of the leaves.

Exp. 136. 2 per cent. oil and 0·5 per cent. soft soap.

Four leaves sprayed. The emulsion proved fungicidal, the patches being semi-obliterated. No injury was caused. The patches on the control (unsprayed) leaves remained densely powdery.

Exp. 174. 1 per cent. oil and 0·5 per cent. soft soap.

Four leaves sprayed, the leaves at the same nodes being sprayed with 1 per cent. sesame oil and 0·5 per cent. soft soap (Exp. 175, see above). The rape-oil emulsion proved non-fungicidal, but with distinct checking action, so that for some of the patches it was almost fungicidal, the sesame-oil emulsion proved fungicidal (super-fungicidal).

Exp. 196. 0·5 per cent. oil and 0·5 per cent. soft soap.

Seven leaves sprayed. The emulsion proved non-fungicidal, although there was distinct checking action, so that for some patches it was almost fungicidal. The patches on the control unsprayed leaves remained densely powdery.

Exp. 169. 2 per cent. oil and 0·4 per cent. Agral W.B.S.

Five leaves sprayed. The emulsion proved fungicidal for the powdery stage, but not for the "hump" stage.

Exp. 209. 3 per cent. rape oil and 0·5 per cent. soft soap.

A stem, some three feet high, and all its leaves (on both surfaces) and growing point were thoroughly sprayed. On the sixth day serious "scorching" injury was visible on one pair of very young leaves, and slight injury on the tips of some of the older leaves; the growing tip showed no injury. No further injury became visible and the tip of the stem grew on normally.

Rape oil is therefore fungicidal at 2 per cent. when emulsified with 0·5 per cent. soft soap, and is non-fungicidal at 1 per cent.

Olive oil.

Exp. 185. 2 per cent. oil and 0·5 per cent. soft soap.

Five leaves sprayed. The emulsion proved fungicidal, the patches being obliterated. The "hump" stage was also killed. No injury was caused.

Exp. 177. 1 per cent. oil and 0·5 per cent. soft soap.

Eight leaves sprayed; the opposite leaves at the same nodes were sprayed with 1 per cent. peach-kernel oil and 0·5 per cent. soft soap (Exp. 176, see below). The olive-oil emulsion proved fungicidal, even for the "hump" stage.

Exp. 193. 0·5 per cent. oil and 0·5 per cent. soft soap.

Five leaves sprayed; the opposite leaves at the same nodes were sprayed with 0·5 per cent. soft soap alone. The olive-oil emulsion proved fungicidal, the patches on the leaves sprayed with the soft soap alone became densely powdery again by the sixth day.

Exp. 202. 0·25 per cent. oil and 0·5 per cent. soft soap.

Five leaves sprayed. The emulsion was not quite fungicidal, most of the patches re-developing a few scattered or clustered conidiophores.

Exp. 210. 1 per cent. oil and 0·5 per cent. soft soap.

A stem, some 3 ft. high, and all its leaves (on both surfaces) and the growing point were thoroughly sprayed. No injury resulted.

Olive oil, with 0·5 per cent. soft soap, is fungicidal at 0·5 per cent. concentration, and not quite fungicidal at 0·25 per cent. No injury to the young growing hop plant was caused by a 1 per cent. olive-oil emulsion containing 0·5 per cent. soft soap.

Peach-kernel oil.

Exp. 166. 4 per cent. oil and 0·5 per cent. soft soap.

Five leaves sprayed. The emulsion was fungicidal, the patches being obliterated. No injury was caused.

Exp. 137. 2 per cent. oil and 0·5 per cent. soft soap.

Six leaves sprayed. The emulsion proved fungicidal.

Exp. 176. 1 per cent. oil and 0·5 per cent. soft soap.

Eight leaves sprayed; the opposite leaves were sprayed with 1 per cent. olive oil and 0·5 per cent. soft soap (Exp. 177, see above). Both emulsions were fungicidal.

Exp. 190. 0·5 per cent. oil and 0·5 per cent. soft soap.

Five leaves sprayed. The emulsion proved to be non-fungicidal, although there was distinct checking action, so that for some patches it was almost fungicidal.

Peach-kernel oil is therefore fungicidal at a concentration of between 0·5 and 1·0 per cent., when 0·5 per cent. soft soap is used as the emulsifier.

Castor oil.

Exp. 197. 4 per cent. oil and 1 per cent. soft soap.

Four leaves sprayed. The emulsion proved fungicidal. No injury was produced.

Exp. 138. 2 per cent. oil and 0·5 per cent. soft soap.

Four leaves sprayed. The emulsion proved to be non-fungicidal, although there was a distinct checking action, so that many patches developed only a few scattered conidiophores.

Exp. 195. 4 per cent. oil and 0·4 per cent. Agral W.B.S.

Five leaves sprayed. The action was very uneven; on some leaves all the patches, including those in the "hump" stage, were killed, while on other leaves some of the patches survived and produced clusters of conidiophores. No injury was produced.

Castor oil, emulsified with soft soap, is therefore fungicidal at a strength between 2 per cent. and 4 per cent.

With regard to the above vegetable oils, it must be pointed out that, as only one sample of each oil was used, further experiments must be made to determine the general applicability of the results to other samples. It is possible, for instance, that the inferior fungicidal properties of the rape oil used may be associated with its high acid value.

SUMMARY.

The following substances proved fungicidal for the conidial stage of the hop mildew (*Sphaerotheca Humuli*):

(1) Liquid paraffin at 2 per cent. emulsified with soft soap. The spray is harmless, or dangerous, to foliage according to the conditions (probably temperature) in the greenhouse.

(2) Medicinal paraffin, emulsified with 0·75 per cent. castor-oil soap, is not quite fungicidal at 3 per cent.

(3) "Summer solol," a proprietary mineral-oil preparation containing 61·6 per cent. by weight high-boiling petroleum oils, is fungicidal at a concentration between 2·5 and 3 per cent. when 0·5 per cent. soft soap is added to confer satisfactory spreading properties. At this concentration no injury to the leaves was caused.

(4) "Volck," a proprietary petroleum-oil preparation containing 80·0 per cent. by weight mineral oils, is fungicidal at 2·5 per cent. with either 0·5 per cent. soft soap or 0·5 per cent. Agral I. Injury may be caused under certain conditions.

(5) Sternol insecticide, a petroleum-oil preparation containing 75·3 per cent. high-boiling petroleum oils, is almost fungicidal at 2·5 per cent., with 0·5 per cent. soft soap.

(6) High-boiling neutral tar oils, whether applied in the form of the two-solution Long Ashton wash, modified Long Ashton wash, or emulsified with castor-oil soap, are toxic to the hop foliage at concentrations greater than 0·25 per cent. and, at lower, are non-fungicidal. Removal of tar acids and crude bases does not prevent foliage injury.

(7) Dekalin at 2 per cent., emulsified with 0·5 per cent. soft soap or 0·5 per cent. Agral I, is non-fungicidal and non-injurious to the foliage.

(8) Tetralin at 2 per cent., emulsified with 0·5 per cent. soft soap or 0·5 per cent. Agral I, is injurious to the foliage.

(9) Cottonseed oil, with 0·5 per cent. soft soap, appears to be fungicidal at a strength between 0·25 and 0·5 per cent.[1] With 0·25 per cent. Agral W.B., cottonseed oil is fungicidal at 0·25 per cent.

(10) Sesame oil, with 0·5 per cent. soft soap, is fungicidal at a strength between 0·5 and 0·25 per cent. With 0·4 per cent. Agral W.B.S., sesame oil is fungicidal at 0·5 per cent. At 4 per cent. sesame oil causes no injury to the young growing hop plant.

(11) Rape oil, with 0·5 per cent. soft soap, is fungicidal at 2 per cent. and non-fungicidal at 1 per cent.

(12) Olive oil, with 0·5 per cent. soft soap, is fungicidal at 0·5 per cent. and not quite fungicidal at 0·25 per cent. At 1 per cent. it causes no injury to the young growing hop plant.

(13) Peach-kernel oil, with 0·5 per cent. soft soap, is fungicidal at a strength between 0·5 and 1·0 per cent.

(14) Castor oil, with soft-soap solutions, is fungicidal at a strength between 2 per cent. and 4 per cent.

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[1] In one anomalous case (Exp. 198*) a strength of 1 per cent. failed to kill all the patches (see above, p. 652).

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THE PERIODS OF EMBRYONIC GROWTH IN CATTLE.

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(With Three Text-figures.)

THE PROBLEM.

MANY authors have studied the post-natal growth of cattle (Ecles[7], Feldmann[8], Gärtner[10], Moulton, Trowbridge and Haigh[16, 17], Brody[4, 5], Fischer[9], Schmalhausen[19, 20] and many more).

On the other hand, embryonic growth has been studied only by a few (Hammond[11], Bergmann[1] and a few others), although Maligonoff[14, 15] pointed out the great importance of studying embryonic development.

One of the present authors (Kislovsky[13]) has presented to the Russian Zoological Congress in Kief, during the summer 1930, a paper dealing with the growth of calves as influenced by breeding and feeding.

His material consisted of nearly 800 calves of fifteen different breeds, which have been under experiment in different countries and was drawn partly from published records, partly from data as yet unpublished. His study led him to doubt the statement of Brody that there is in cattle only one period of "self-accelerating growth" during extra-uterine life (from birth till about the fifth month). Most of his material indicated two periods with a sudden break between them. In several individuals and even whole groups there were as many as three periods. Such, for instance, was the case with the brown Polessky breed (data from the agricultural experiment station, Novozibkovo).

The American dairy breeds alone showed the exceptional behaviour of having only one period of growth at a constant rate.

On analysing Brody's[5] own data about the Shorthorn breed, we also have a second break in the growth constant at the age of about 1 month.

We see in Table I an abrupt fall of the growth constant from $K = 0.48$ to something about $K = 0.24$. In a similar manner we find in Moulton's[16] data for cross-bred Shorthorn-Herefords (full-fed) during

the first month, $K = 0.5223$; during the second, third and fourth respectively 0.2604, 0.2053 and 0.2283.

Table I. *Normal growth of a Shorthorn heifer.*

Age from birth (weeks)	Weight (kg.)	Growth constant K per month (4 weeks)
0	33.1	
4	53.5	0.4802
8	60.4	0.1213
12	79	0.2684
16	102	0.2556
20	121.5	0.3378

In order to explain this discrepancy Kislovsky advanced the hypothesi sthat the American dairy breeds (Holstein-Friesians, Jerseys and Ayrshires) were quicker developing during their intra-uterine life and passed one period of "metamorphosis" *in utero*, which the majority of other breeds experienced only after birth. Breeds which have suffered severely from under-development during many generations ("neotenic" or "embryotenic" according to the nomenclature of Prof. Maligonoff (15)), as the quoted brown Polessky breed, did not pass even two periods of "metamorphosis" *in utero*.

ORIGINAL MATERIAL AND ITS ANALYSIS.

To test this hypothesis it was held important to collect observations about the periodicity of embryonic growth for different breeds of cattle. On this suggestion the junior author (B.A.L.) spent some time procuring embryological material of known age of the Domshino cattle (an underdivision of the Jarosslav breed). He intends to analyse this material thoroughly. In the present paper the authors will be concerned only with the question of the periodicity of growth during embryonic development.

There are in our possession fifteen calf embryos ranging in age from 40 to 270 days which were weighed and measured immediately after they were received subsequent to the slaughter of their mothers.

The data are set down in Table II.

Table II. *Weight of embryos at different ages.*

Age (days)	Weight (gm.)	Age (days)	Weight (gm.)
40	2.1	135	1,228
53	9.2	165	3,700
60	56	180	6,700
78	67	210	11,400
90	161.5	240	15,200
96	327	255	18,200
105	610	270	30,000
120	645.7		

Putting these data on arith.-log. paper by the method devised by Brody (4), we obtain the following figure (see Fig. 1).

These data show clearly the existence of three abrupt breaks in the growth curve: three "periods of metamorphosis."
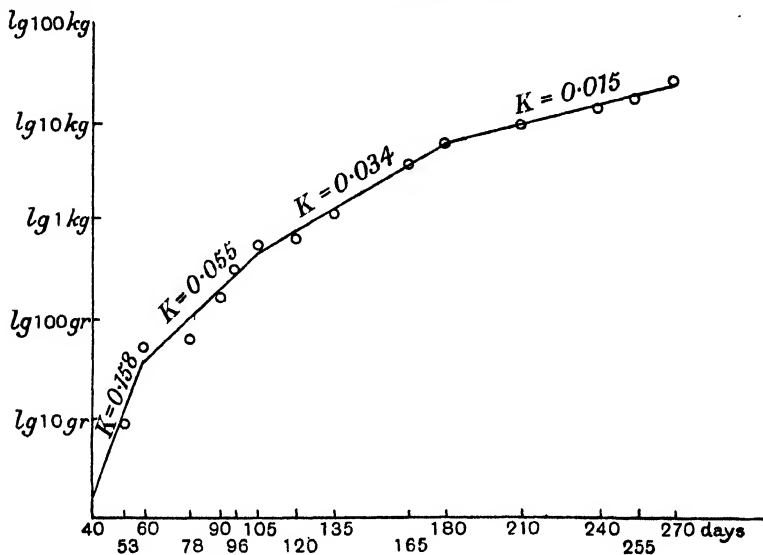
Fig. 1.

Between two breaks the growth rate goes on at a steady level with minor fluctuations. In Table III are given the calculated growth rates K for each interval (column 2 of the table) and the growth rate constants between two breaks (column 3).

Table III. *Growth rate of embryos.*

1	2	3
Period	The growth constant K	The most probable K between two breaks
40– 53	0·11364⎫	
53– 60	0·25802⎭	0·15773
60– 78	0·00996⎫	
78– 90	0·07330⎪	
90– 96	0·11758⎬	0·05480
96–105	0·06928⎭	
105–120	0·00381⎫	
120–135	0·04284⎪	
135–165	0·03676⎬	0·03394
165–180	0·03959⎭	
180–210	0·01771⎫	
210–240	0·00959⎪	
240–255	0·01202⎬	0·01506
255–270	0·03331⎭	

The last number was calculated by the method of least squares. The constant K is calculated for the time interval of 1 day.

From these data we may conclude that the course of intra-uterine growth of cattle proceeds on the same lines as that of the rat, as has been demonstrated by Brody on the material of Donaldson, Dunn and Watson, or MacDowell's data on the mouse[1].

If we compare the growth rate of a calf of the same breed just before birth with that after birth, we see that no important change takes place. A Domshino calf grows at the rate $K = 0.01506$ a day before birth (Table III), and during its extra-uterine first month at a rate $K = 0.0145$ (for different individuals from $K = 0.0122$ to $K = 0.0165$). The rather violent changes that take place in the external conditions of existence do not materially affect the growth rate. This is in full accordance with Robertson's[18] data about the growth of man and in contrast with Schmalhausen's theoretical conclusions as to the rather marked influence of external conditions upon the growth rate. On more thoroughly studied material, with weighing every week, we may see that this violent change in the conditions of existence (birth) has a checking influence upon the growth rate just after birth, which is followed by a compensatory increasing growth rate. For instance, in the polled breed of Smolensk (data of Borissenko[3]) we have a weekly growth rate just after birth 0.0752 and in the three following weeks 0.1485, 0.1382 and 0.1670 respectively. The same compensatory overgrowth after a temporary slowing of the growth rate has been observed by Robertson (*loc. cit.* Figs. 3 and 4 on pp. 29 and 30) in man.

Our general conclusion may be stated as follows: *the rate of growth and even more especially the periodicity of growth are primarily dependent on the constitutional stage of the developing organism and not on the external conditions.*

ANALYSIS OF PUBLISHED RECORDS.

It is of some interest to analyse on the same lines material of other authors drawn from published data. Hammond[11] published interesting materials about the intra-uterine growth of grade Shorthorns. His material has the advantage over ours that all calves are from heifers calving for the first time and of about an equal age. The possible influence of age and of different degrees of lactation in the mother on the developing embryo is thus eliminated.

These data plotted on arith.-log. paper show the picture already

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[1] *Journ. Gen. Physiol.* (1927), **11**, quoted after Schmalhausen (19).

familiar to us of growth during the self-accelerating phase with several changes in rate (see Fig. 2).

The first break takes place at about 56 days after conception, the second at about 112. These two periods correspond fairly well with our data for the Domshino cattle (compare Figs. 1 and 2).
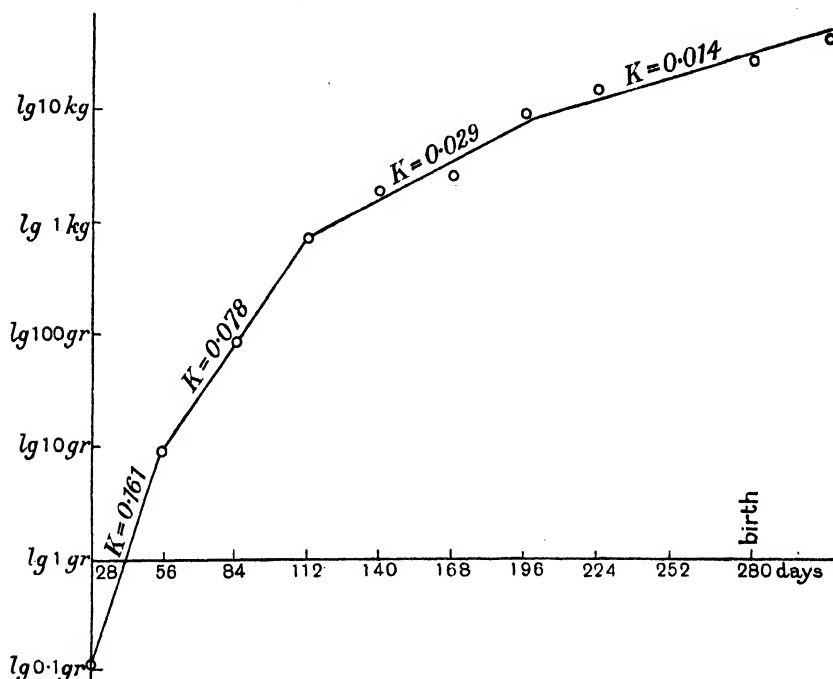
Fig. 2.

But there is no evidence of our third break in the grade Shorthorns at the age of 180 days. Hammond's data give no material for the last stages of embryonic growth. If we take Ecles' (7) data of birth weight of Shorthorns—33·1 kg.—that will give (on the assumption of birth taking place on the 284th day according to Hansen (12)) a growth rate of $K = 0.0114$ per day up to the moment of birth. The growth rate for the first month of extra-uterine growth goes on with the rate of $K = 0.01715$ per day.

We may conclude, therefore, that the rate of growth does not materially change at birth, and it is probable that there is a break in the growth rate of the Shorthorn calf at 196 days (or a little later) after conception.

In order to have data on a typical dairy breed we may take Bergmann's[1] data (quoted from Hammond) concerned with the embryonic growth of the "spotted Niederungsbreed" (Hammond, *loc. cit.* p. 133), of a breed nearly related to the Holstein-Friesians. Bergmann's data show the following picture on arith.-log. paper (see Fig. 3).

Table IV. *Growth rate of grade Shorthorn during intra-uterine life (after Hammond).*

Period	Weight at beginning (gm.)	K	K
28– 56	0·1	0·16149	0·16149
56– 84	9·2	0·08024	
84–112	87	0·07548	0·07786
112–140	720	0·03332	
140–168	1,830	0·01389	0·02998
168–196	2,700	0·04809	
196–224	10,380	0·01711	—
224	16,760	—	—

We may perceive here also only two characteristic breaks in the growth curve.

Table V.

Period	Weight at beginning (gm.)	K	K
56– 84	7	0·11694	0·11694
84–112	185	0·04488	
112–140	160	0·05019	0·04754
140–168	2,650	0·03093	
168–196	6,300	0·01504	0·02278
196–224	9,600	0·02494	
224–284	19,300	—	—

In order to have some comparable data for the growth rate during the last period of intra-uterine growth and the first period of extra-uterine, we may use Fischer's data[9] (quoted from Bogdanoff[2]).

An Ostfriesland (East Friesian) heifer weighs 35·125 kg. at birth and 70·5 kg. at the age of 3 months.

These data would give a growth rate of $K = 0·0100$ for the period from 224 days after conception to birth, and $K = 0·00785$ for the growth rate after birth.

Nor does birth disturb the growth rate to a marked degree in this instance. The small decline during the post-natal life is probably due to the fact that a break may have taken place during the last period of intra-uterine life.

An unmistakable break did occur during the time after 224 days before birth ($K = 0·0249$ and $K = 0·0100$) (see Table V and Fig. 3).

. If we continue both lines (that with $K = 0·0249$ and that with

$K = 0.0100$) on the sketch till they cross each other, we will have the most probable point of the break at something like 238 days after conception. On Fig. 3 these lines are shown by dots.

The growth of a "normal" Holstein-Friesian from Ecles' data shows after birth the growth rate of $K = 0.0106$ per day, or nearly the same rate as the Ostfriesland breed in Fischer's data.
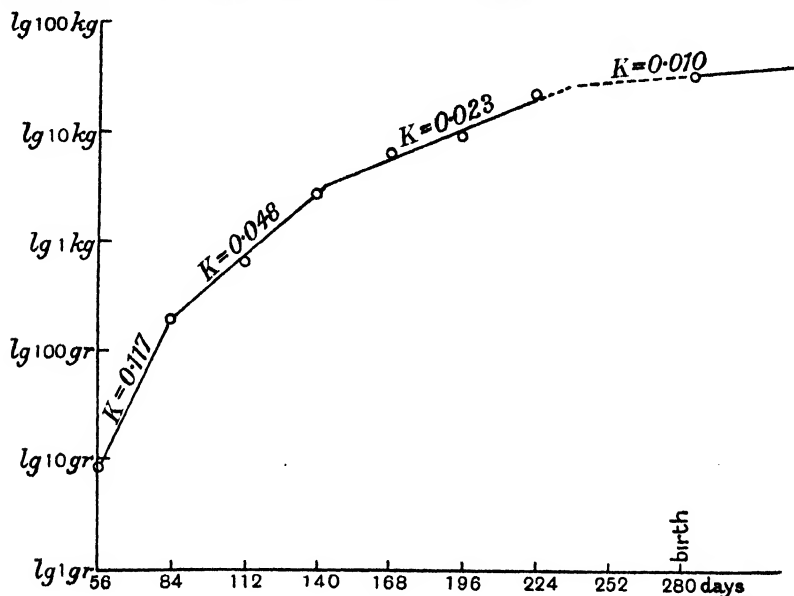
Fig. 3.

A COMPARISON OF DIFFERENT BREEDS.

We may now tabulate our data for our three breeds in the following form (Tables VI and VII).

Table VI. *The corresponding breaks in the growth rate of three breeds.*

Breed	1	2	3	4	5 and last
Domshino	60	105	180	324	441
Shorthorns	56	112	196	314	434
Niederungsbreed	Before 56 data not available	84	140	238	396

Table VII. *The rate of growth during the corresponding periods.*

Breed	1	2	3	4	5
Domshino	0.15773	0.05480	0.03394	0.01506	0.00819
Shorthorns	0.16149	0.07786	0.02998	0.01355	0.00702
Niederungsbreed	—	0.11694	0.04754	0.02278	0.01000

Table VI shows that the rather primitive Domshino cattle reaches most of the "breaks" considerably later than the specialised beef and dairy breeds. The dairy breed reaches the corresponding stages earlier than the beef breed.

The dairy breed grows at the corresponding stages quicker than both the primitive and the beef breed, which corresponds with its earlier attainment of these points of development.

The length of different periods is not the same in the different breeds. We have not a simple acceleration of the whole process of development in the specialised breed as compared with the primitive one: we have a differential prolongation in differently specialised breeds.

Table VIII. *Length of the respective periods.*

	A primitive breed (Domshino)		A beef breed (Shorthorns)		A dairy breed (sp. Niederungsbreed)	
Period	Length in days	%	Length in days	%	Length in days	%
*1	60	100	56	91·5	Less than 56	More than 93
2	45	100	56	124	Less than 24	Less than 53·5
3	75	100	84	112	56	75
4	114	100	118	82	98	68
5	117	100	121	103·5	158	135

* Period 1 is certainly not homogeneous: it no doubt consists of several periods, but data thereon are lacking.

There are shown in Table VIII the respective lengths of the different periods in days. To make comparison easier all data are given in per cents. of the primitive breed. It may be seen that whereas in the beef breed periods 2 and 3 exceed those of the primitive breed, period 3 is markedly shorter in the dairy breed (for 2 we have no reliable data as the beginning of that period is unknown). On the other hand, period 5 is markedly longer in the dairy breed and about the same length in the beef breed as in the primitive one. *The evolution of several breeds of cattle, that leads them to assume different specialised features, left marked differences in the course of their embryonic development.*

As to what the cause of these changes in the development may be we can only make some suppositions. According to Brody's views (4, 6) the break in the growth rate must be conditioned by the fact that certain growth-inhibiting substances reach (and exceed) certain threshold values. This may be attained either by acceleration of the formation of these growth-inhibiting substances, or by the increase of the susceptibility of the growing protoplasm to the same concentrations of the growth-inhibiting substances.

Both conditions may be produced either by changes occurring in the hereditary constitution of the developing organism (presumably mutations of genes) or by the effects of changes in the circumstances of development.

On the former assumption these differences in the course of development must follow the Mendelian laws in cases of cross-breeding; on the latter, experiments of transferring fertilised ova from one breed to another will show the periodicity of growth in accordance not with the real mother but with the foster mother. Results of reciprocal crosses must show on the last assumption purely matroclinal characters of growth.

Our material does not allow us to solve these questions.

Conclusions.

1. The authors claim to have demonstrated that there are, after 40 days from conception, five periods of growth in the calf during the self-accelerating phase of growth.

2. These periods are of different length in different breeds.

3. Some breeds, as the well-developed dairy breeds, pass the last break in the rate of growth before birth; all others have one, some as much as two, after birth.

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THE COMPOSITION OF MARE'S MILK.

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THE composition of the milk of the cow has naturally attracted much attention because of its importance as a human food. A knowledge of the chemistry of the milk of other domestic animals is, however, unfortunately defective, notwithstanding the fact that normal milk is an essential factor in the satisfactory rearing of the young.

While, to a large extent, the selection and breeding of cows have been controlled by a desire to improve either the quality or quantity, or both, of the milk, no such consideration has hitherto influenced breeders of horses. The suitability of the milk of mares for the rearing of healthy foals has seemingly been taken for granted.

Not infrequently complaints are made that a suckling animal is unable to rear her offspring satisfactorily, and this is the case occasionally with brood mares. Samples of mare's milk in cases of this nature having been received in the laboratory for examination, search of the literature for information on the normal composition naturally followed. While no information seems to be available regarding the malnutrition of foals that results from the secretion of abnormal milk by the dam, several publications deal with the normal composition of mare's milk, though some of them are reports of analyses made before modern improvements in technique were adopted. Almost all work published hitherto deals with the milk of German, French or Russian mares, and it seems strange that the economic importance of British horses has not hitherto led to a detailed examination of the milk of the mares of our own breeds.

The importance of a clear conception of the composition of mare's milk may be emphasised by the mention of the well-known difficulty in rearing orphan foals satisfactorily on substitutes, a difficulty that is probably greater than that with the young of any other domestic animal.

For purposes of comparison, the recorded work of previous investigators may be grouped conveniently in the form of a table (Table I). These records of analyses have been collected from the literature and, so far as can be ascertained, comprise all former observations.

Table I. *The percentage composition of mare's milk as stated*
by previous observers.

	Total solids (%)	Protein (%)	Fat (%)	Sugar (%)	Ash (%)
Schrodt (1)	8·85	1·50	1·27	5·75	0·37
Becquerel and Vernois (2)	9·57	3·34	2·44	3·28	0·52
Fleischmann (3)	9·30	2·00	1·20	5·70	0·40
König (4)	9·42	2·05	1·14	5·87	0·36
König (5)	10·12	2·20	0·54	6·97	0·41
Möser and Soxhlet (6)	7·51	1·69	0·65	4·72	0·29
Petersen and Höfker (7)	9·82	2·14	0·61	6·73	0·35
Vieth (8)	9·94	1·89	1·09	6·65	0·31
Monvoisin (9)	10·80	2·30	2·00	5·60	0·40
Hildebrand (10)	9·98	2·87	0·98	6·37	0·45
Wynter Blyth (11)	11·20	2·70	2·50	5·50	0·50
Average*	9·78	2·18	1·29	5·93	0·38
Maximum	11·20	3·34	2·50	7·26	1·20
Minimum	7·47	1·33	0·12	3·28	0·26

* It is recognised that as the number and particulars of the analyses made by some of these workers is not known, the "average" figures here computed are not true averages but really represent the mean of the.averages previously obtained.

Of the records given in Table I two are of particular interest. Vieth's investigation attracts special attention because the samples analysed were obtained from mares that were accustomed to being hand-milked, being kept for the purpose of providing milk for making koumiss. A stud of Tartary horses including fifteen mares was brought from the Russian Steppes in 1884 and exhibited at the International Health Exhibition for the purpose of advertising koumiss and condensed mare's milk. The mares were from five to six years old and had foaled about five months previously. In view of the great difficulty in obtaining samples of mare's milk representative of twenty-four hours' secretion—a difficulty that does not exist with the cow—the procedure followed with these mares is of interest. From eight o'clock in the morning to six o'clock in the afternoon the foals were kept from their dams, which were hand-milked every hour during this period. Thus Vieth was able to obtain representative samples and avoid the risk of analysing milk that had been retained in the udder under pressure with consequent fat depression.

Vieth found remarkably little variation in the milk of the fifteen mares; a uniformity in composition that may be accounted for by the frequency and regularity with which the milk cisterns were emptied, and also probably in part by the fact that the mares were selected, were of one breed and were fed and managed in an identical and uniform manner.

Search of literature has brought to light only one record of the analysis of British mare's milk. The late A. Wynter Blyth, in his well-known text-book[11], gave the chemical composition of mare's milk that is quoted in Table I. His son[1], Mr M. Wynter Blyth, states that the figures were obtained by the analysis of milk of mares in North Devon (Barnstaple). The results are of special interest because Wynter Blyth found an appreciably higher percentage of total ash than is recorded for the average of the continental mares, to which reference has previously been made. His ash estimation agrees with those obtained in the present investigation for heavy breeds of horses. This will be further discussed when the mineral composition of milk is considered in detail.

THE PRESENT INVESTIGATION.

During the foaling season of 1926-7 samples of mare's milk were sent to the laboratory with requests for reports on their composition, it being suspected that the dam's milk did not agree with the foals. A considerable amount of variation having been found in these early samples, more were collected and analysed at the writer's request by Prof. R. G. Thin, B.Sc., F.I.C. These are referred to in this paper as A 1 to A 25.

Because, other than that recorded by Wynter Blyth, no analyses of the milk of British breeds of horses could be found with which to compare the results obtained, it was decided to extend the investigation; one hundred and forty-two samples were obtained and analysed, the composition of which forms the basis of the discussion in this paper.

The work undertaken was confined to the determination of the total solids, protein, fat, sugar and ash.

The aims of the investigation were as follows:

(a) To ascertain if the average composition of the milk of British mares is similar to that of continental mares.

(b) To determine what differences, if any, exist.

(c) To ascertain if the composition of the milk of British mares is the same for each breed.

(d) In cases where the mares were suckling unthrifty foals, to attempt to ascertain if the composition of the milk could be held accountable for the failure of the foals to thrive.

Since numerous samples of colostrum were also received, observations on their composition are noted.

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[1] Personal communication.

TECHNIQUE.

Protein. The total protein was estimated by Kjeldahl's method, the nitrogen being multiplied by 6·33. Estimations were made in duplicate, 10 c.c. of milk being used in each estimation.

Total solids. 10 c.c. was evaporated to dryness and kept in a water oven until constant weight had been reached.

Ash. The dried residual solids, after having been burnt off in open air over a low bunsen flame, were ashed in a muffle furnace, the back of the furnace just showing a dull red heat.

Fat. The estimations recorded were obtained by the Werner-Schmid method, 10 c.c. of milk being used from each sample. In nearly all cases check tests were made by the Gerber centrifuge method.

Sugar. It being found impracticable to estimate the sugar directly in anything but a very small proportion of the samples, the sugar percentages given in the tables are determinations "by difference."

RESULTS.

Of the samples supplied from one hundred and forty-two mares of different breeds, thirty-eight were collected during the first three days after parturition. There were therefore one hundred and four from which to establish the average composition of the milk of British mares, and thirty-eight from which to observe the composition of colostrum. The results obtained by analysis, together with the breed and age of the mare (when known) and the number of days that had elapsed after foaling before the sample of milk was collected, are collated in Table II.

The representatives of each breed are as follows: 62 Clydesdales, 8 Shires, 14 Thoroughbreds, 4 Ponies and 1 Hunter. It is unfortunate that among the one hundred and four results of analyses given in Table II some are of samples obtained from mares the breed of which was not stated. While this necessarily lessens the reliability of the comparisons between the different breeds, the residual facts are of moment, and indicated herewith.

A comparison of the results obtained during the present investigation and the figures given by all previous observers may be made by an examination of Table III, in which analyses have been reduced to average, maximum and minimum.

Table II. *The comparison of one hundred and four samples of milk (non-colostral) from various breeds.*

No.	Breed	Age (years)	Days after foaling	Total solids	Protein	Fat	Sugar	Ash
3	S.	7	20	9·74	1·92	0·38	6·80	0·64
4	C.	10	5	11·11	3·88	0·31	6·17	0·75
7	L.D.	?	12	13·01	2·03	3·49	6·96	0·53
8	C.	?	9	8·97	2·94	1·40	3·93	0·70
9	C.	?	10	18·74	7·60	7·88	2·31	0·95
10	C.	7	20	10·39	2·41	0·42	7·02	0·54
11	H.	9	10	12·84	1·52	3·40	7·11	0·81
12	C.	?	9	12·41	3·01	2·23	6·69	0·48
14	C.	6	8	10·01	2·29	0·42	6·81	0·49
15	C.	5	40	13·23	2·85	3·50	6·33	0·55
17	?	?	28	9·98	1·94	1·05	6·53	0·46
18	C.	8	11	10·12	2·20	0·45	6·93	0·54
19	C.	12	10	11·47	2·80	1·68	6·42	0·57
20	T.	18	48	12·26	2·42	3·35	6·09	0·40
21	T.	8	58	10·96	2·09	2·06	6·36	0·45
24	T.	8	82	10·01	2·08	0·59	6·97	0·37
25	C.	8	7	10·72	2·73	1·68	5·71	0·60
26	S.	?	30	10·90	2·19	1·47	6·69	0·55
27	C.	20	35	11·68	2·26	1·99	7·01	0·42
28	C.	8	13	11·03	2·52	1·13	6·88	0·50
29	T.	10	35	9·26	1·99	0·25	6·73	0·29
32	C.	7	30	8·80	2·83	1·19	4·24	0·54
33	C.	?	17	10·21	2·46	0·39	6·96	0·40
34	C.	?	18	12·39	2·80	0·94	8·17	0·48
35	C.	?	43	11·79	2·59	2·21	6·49	0·50
36	C.	?	24	10·93	2·04	1·71	6·77	0·41
38	C.	?	42	10·61	2·66	0·45	7·05	0·45
39	C.	?	47	10·67	2·31	0·44	7·48	0·44
40	C.	?	19	12·42	3·38	2·49	5·99	0·56
41	T.	?	100	10·34	0·55	0·73	8·78	0·28
42	C.	7	23	11·40	2·89	1·81	6·18	0·52
43	S.	6	19	10·55	2·47	0·09	7·50	0·49
44	S.	?	13	10·44	2·08	0·57	7·26	0·53
45	C.	14	6	11·43	3·23	0·67	6·99	0·54
48	C.	13	8	12·97	2·76	2·28	7·37	0·56
49	?	7	56	11·15	2·41	1·77	6·52	0·45
50	C.	7	56	11·58	3·28	1·51	6·25	0·54
51	F.P.	?	63	10·46	2·28	0·66	7·08	0·44
52	S.	6	35	9·36	2·41	0·20	6·27	0·48
55	T.	19	Weaning	8·64	2·08	0·35	5·78	0·43
59	?	?	270	5·93	1·65	0·77	3·13	0·48
60	T.	9	90	9·61	1·71	0·33	7·26	0·31
63	T.	?	34	11·64	2·09	2·70	6·42	0·43
66	C.	5	6	11·09	2·68	1·44	6·30	0·67
69	C.	5	5	11·59	2·60	2·10	6·26	0·63
71	C.	5	4	10·95	2·90	1·72	5·73	0·60
74	C.	5	7	9·97	2·72	0·13	6·57	0·55
76	T.	?	7	9·71	3·48	1·71	3·82	0·70
77	S.	9	270	13·49	5·44	2·57	4·87	0·51
81	C.	7	14	11·24	2·41	1·95	6·23	0·65
83	C.	?	7	11·71	2·91	2·25	6·02	0·53
84	T.	?	?	11·22	2·53	0·29	7·85	0·55
85	L.D.	13	35	10·83	2·65	1·58	6·01	0·59
86	?	?	?	11·49	2·28	2·40	6·34	0·47
87	S.P.	6	14	10·30	2·48	0·90	6·65	0·35

Table II (*cont.*)

No.	Breed	Age (years)	Days after foaling	Total solids	Protein	Fat	Sugar	Ash
88	C.	?	9	8·14	3·04	1·36	3·06	0·68
89	S.P.	6	14	9·62	1·90	0·50	6·91	0·31
92	C.	13	10	13·02	4·62	2·32	5·44	0·64
93	P.	16	21	10·77	2·53	2·40	5·42	0·42
95	C.	Aged	8	11·81	3·10	2·21	5·88	0·62
96	C.	?	7	11·92	3·04	2·00	6·33	0·55
97	C.	6	21	9·02	1·71	0·26	6·62	0·43
98	T.	18	30	12·05	1·52	1·82	8·29	0·42
100	S.	?	4	10·83	3·57	0·65	5·89	0·72
101	C.	5	14	10·19	2·84	0·40	6·42	0·53
102	C.	10	13	11·26	2·60	1·89	6·27	0·50
103	S.	4	28	11·87	3·86	2·20	5·40	0·41
107	C.	4	30	10·85	2·65	1·25	6·49	0·46
109	C.	?	8	10·27	2·72	1·30	5·71	0·54
110	C.	7	27	10·74	2·60	1·70	5·95	0·49
112	T.	?	90	10·97	1·96	0·57	8·07	0·37
113	C.	7	6	12·13	3·17	1·70	6·65	0·61
115	C.	?	30	10·11	3·10	1·80	4·78	0·43
116	C.	?	75	10·38	2·34	1·30	6·45	0·29
117	C.	?	45	9·93	2·46	0·54	6·45	0·48
119	T.	?	105	10·59	1·93	1·24	7·14	0·28
120	C.	5	9	12·81	3·61	2·00	6·66	0·54
121	T.	?	90	10·40	1·90	1·14	7·03	0·33
122	?	3	21	9·88	2·41	0·54	6·42	0·51
123	T.	9	60	10·73	2·41	1·10	6·80	0·42
124	C.	24	14	12·12	3·29	2·14	6·09	0·60
125	C.	9	120	9·16	2·15	0·28	6·45	0·28
A 1	C.	7	60	12·58	3·35	2·65	6·08	0·50
A 2	C.	?	42	10·42	3·04	0·75	6·06	0·57
A 3	C.	?	7	8·77	2·28	0·85	5·06	0·58
A 4	C.	?	13	9·39	2·02	2·75	4·01	0·61
A 5	C.	?	14	11·09	2·53	1·50	6·59	0·47
A 6	C.	?	12	9·80	2·47	1·52	5·19	0·62
A 7	C.	?	14	11·40	2·47	2·60	5·84	0·49
A 9	H.	Aged	21	13·93	3·23	4·40	5·86	0·44
A 10	?	?	16	12·42	3·23	5·30	3·15	0·74
A 11	C.	?	31	9·87	2·47	0·70	6·17	0·53
A 12	C.	?	40	10·09	2·22	0·75	6·64	0·48
A 13	C.	?	?	13·03	3·17	3·60	5·79	0·47
A 14	C.	?	35	10·53	2·47	1·85	5·60	0·61
A 15	C.	14	10	8·95	2·91	0·40	5·19	0·45
A 16	C.	?	?	11·41	3·79	1·30	5·70	0·62
A 17	C.	?	?	12·55	2·53	3·30	6·34	0·38
A 18	C.	?	?	10·67	2·59	2·00	5·68	0·40
A 19	C.	?	?	11·42	3·61	1·70	5·49	0·62
A 22	C.	?	7	10·96	3·48	1·09	4·80	0·59
A 23	H.	?	75	11·43	2·60	2·25	6·15	0·43
A 24	H.	?	30	8·70	3·42	2·85	1·65	0·75
A 25	?	?	?	12·58	3·35	2·65	6·08	0·50
		Average		10·96	2·69	1·59	6·14	0·51
		Maximum		18·74	7·60	7·88	8·78	0·95
		Minimum		5·93	0·55	0·09	1·65	0·28

C. Clydesdale.	H. Hunter	L.D. Light draught.	S.P. Shetland pony.
S. Shire.	T. Thoroughbred.	P. Pony.	F.P. Fell pony.

Table III.

Previous observations (Table I).

	Total solids (%)	Protein (%)	Fat (%)	Sugar (%)	Ash (%)
Average	9·78	2·18	1·29	5·93	0·38
Maximum	11·20	3·34	2·50	7·26	1·20
Minimum	7·47	1·33	0·12	3·28	0·26

Present investigation (Table II).

Average	10·96	2·69	1·59	6·14	0·51
Maximum	18·74	7·60	7·88	8·78	0·95
Minimum	5·93	0·55	0·09	1·65	0·28

From the above figures it is evident that the average composition of the milk of British mares differs very little from that of continental mares[1].

The averages for protein, fat and sugar agree very closely.

The total solids for British breeds are slightly higher, but the only important difference is in the amount of mineral matter, which, taking the average of all samples examined, is higher than that found in the milk of continental mares.

No appreciable variation in the constituents of the milk is notable in different breeds, except in regard to the mineral matter. The milk of the heavier breeds was found to contain more ash than is present in the milk of the lighter breeds, and is consequently given detailed consideration herein.

MINERAL MATTER.

The average amount of ash in one hundred and four samples of milk, free from colostrum, from mares of different breeds was found to be 0·51 per cent. This is considerably greater than the 0·38 per cent. which is the average of the continental figures.

A comparison of the ash content of the milk of the different British breeds seems to indicate that the amount of mineral matter in mare's milk increases with the increase in weight of the breed, as the following table shows:

Table IV. *Percentage of mineral matter in the milk of British breeds.*

Breed	Average weight (lb.)	Ash percentage		
		Average	Maximum	Minimum
Shire	2000	0·54	0·72	0·41
Clydesdale	2000	0·52	0·75	0·28
Hunter	1000 to 1340	0·43	—	—
Thoroughbred	—	0·38	0·55	0·28
Ponies: Shetland	336	0·38	0·44	0·31
Fell	800			

[1] A note concerning the computation of these "averages" is given at the foot of Table I.

With the above average weights should be compared those of German horses, which will be found to be appreciably less than those of Shires and Clydesdales. The average weights of German breeds are as follows:[1]

	lb.
Rhineland draught horse	1760
Hannoverian draught horse	1670
Anhalt draught mares	1550
East Prussian heavy cart horse	1540
Oldenburgh horse	1390
Holstein coach horse	1320

The higher average figures for the ash found during this enquiry ·is therefore possibly due to the large size of Clydesdales and Shires.

In the only record of milk analysis of British mares (that by Wynter Blyth), the percentage of ash is given as 0·50, that is, the same as the average for one hundred and four samples of colostrum-free milk analysed in this investigation.

In this connection, it is well to remember that Bunge, Proescher and Abderhalden(12) have drawn attention to the definite relationship that exists between the rate of growth of animals of different species and the amount of protein and mineral matter in the milk, the rate of growth increasing with the increase in the amount of these constituents, as is shown in the accompanying table.

Table V.

Species	Time required to double weight (days)	Protein in milk (%)	Total ash in milk (%)
Human	180	1·6	0·20
Horse	60	2·0	0·40
Cow	47	3·5	0·70
Sheep	15	4·9	0·84
Pig	14	5·2	0·30
Dog	9	7·4	1·33
Rabbit	6	14·4	2·50

As Henry and Morrison (13) have pointed out the modern pig may double its weight in from 9 to 10 days.

Now there are probably variations in the ash percentage in the milk of different breeds within a species; and if the growth rate of the individual member of a species is constant, notwithstanding variations in size and weight between breeds, then it might be expected that the breed with the greater average weight would have the higher percentage

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[1] The weights of British and German horses were kindly obtained for the writer by the Imperial Bureau of Animal Genetics.

of ash, and *vice versa*. But information on this aspect of nutrition during the early period of extra-uterine life is very meagre and unsatisfactory.

ABNORMAL MILK.

While this investigation was primarily undertaken for the purpose of determining the normal composition of the milk of British mares reared and pastured on British soil, many samples of milk from mares whose foals were not thriving were sent for examination, and it is of interest to examine the records of these cases to see what bearing, if any, the composition of the milk had on the health and general vitality of the foal.

A condition of unthriftiness in suckling offspring may be due to one or more of several causes. For example, the young animal may be born with an inherent weakness or be suffering from a specific disease, such as "Joint Ill"; or, on the other hand, the maternal nutriment may be faulty either in quantity or in quality. It is in connection with the second of these conditions—the quality of the milk—that a study of the analyses may possibly throw some light on the inability, temporary or persistent, of the foal to thrive.

The effect of oestrum.

It is well known that in those animals having an oestral period during lactation the milk may be changed to such an extent as to cause a definite disturbance in the young. Diarrhoea, which may last for several days, is a common symptom of the digestive disturbance that frequently follows the ingestion of the milk taken during this period, and it is well known that such milk may produce digestive troubles in children that drink it (Monvoisin[14]).

A great many observations have been made on the effect of oestrum on the milk of the cow, and all observers agree that no general rule can be laid down as to the manner in which the milk may become changed at this period (Macintosh[15], Ernst[16], Monvoisin[14]). As Ernst expresses it "there are no set influences in one and the same animal, and still less so in different animals." The onset of oestrum may not affect the milk at all, but when it does there is usually a diminution in quantity and an increase in the amount of the total solids (Fascetti and Bertozzie[17]). Rolet[18] found an increase in dry matter and fat in the milk of one cow and the reverse in that of another. Fascetti[19] gives analytical figures of the milk of a cow before, during and after oestrum and shows that in this particular case oestrum produced a decided increase in total

solids, protein, fat and ash, and a scarcely appreciable reduction in the amount of lactose. On the other hand, a distinct diminution in the percentage of fat has been observed by Deschambre and Giniéis[20].

The changes that take place in the composition of milk during oestrum are obviously very variable and appear to depend largely upon the degree of excitability of the animal. Weber[21], for example, produced interesting evidence to show that the presence of a strange milker during the cow's oestrum intensifies the chemical changes. This, it would appear, has an important bearing upon the handling of mares, particularly those of an excitable nature when in heat, and indicates the advisability of keeping them as quiet as possible.

In addition to the facts disclosed by the observations just mentioned there can be no doubt that changes other than those in the amount of protein, fat sugar and ash take place in the milk of some animals. In very excitable cows, for instance, oestral milk is decidedly acid and soon "turns," but whether a similar condition obtains in the milk of mares and sows has not yet been determined. A search of the literature has failed to find any reference to the condition and effects of mare's milk during oestrum.

Oestral milk of mares.

During the present investigation it has fortunately been possible to extend our knowledge of the peculiarities of mare's oestral milk, for several samples have been received, and along with some of them was sent the statement that the foals were indisposed and suffering from diarrhoea. Owing to the fact that the milk was sent by post, in some cases from a considerable distance, it was decided that determinations of the reaction of samples would probably give misleading results, particularly so as mare's milk readily undergoes alcoholic fermentation. Although in some cases steps had been taken to preserve the milk by the use of formaldehyde, thymol, or ice packing, a slight acidity was almost inevitable.

Table VI gives the analyses of those samples drawn during the oestral period that show some degree of departure from the normal. It would appear from these figures that the same changes take place in mare's milk as in cow's milk during oestrum, the fat being the most variable constituent. No. 1 of the table had a remarkably high ash content (1·04 per cent.), and when milk was drawn from the same mare ten days later it was found that the ash had dropped to 0·64 per cent., while the fat had increased from 0·20 to 0·38 per cent.

Table VI. *Percentage composition of abnormal milk secreted during oestrum.*

No.	Solids (%)	Protein (%)	Fat (%)	Sugar (%)	Ash (%)
1	9·21	2·57	0·20	5·4	1·04
11	12·84	1·52	3·40	8·11	0·81
15	13·23	2·85	3·50	6·33	0·55
38	10·61	2·66	0·45	7·05	0·45
74	9·97	2·72	0·13	6·57	0·55
9 A	13·93	3·23	4·40	5·86	0·44
10 A	12·42	3·23	5·30	3·15	0·74

Abnormal fat content.

Apart from variations during the oestral period, some abnormal percentages of fat were found; some being remarkably high and some very low. The following are the ten highest and the ten lowest percentages.

Table VII. *Abnormal fat percentages.*

No.	Period	Highest (%)	No.	Period	Lowest (%)
72	58 hr.	13·13	43	19 days	0·09
78	3 days	12·47	74	7 days	0·13
56	1 day	8·23	52	35 days	0·20
9	10 days	7·88	1	10 days	0·20
31	2 days	5·64	29	35 days	0·25
10 A	16 days	5·30	97	21 days	0·26
9 A	21 days	4·40	125	120 days	0·28
22	36 hr.	3·81	118	13 hr.	0·28
13 A	—	3·60	84	—	0·29
15	10 days	3·50	2	1 day	0·30

Abnormally high fat content.

In seven cases out of ten showing the highest percentages of fat, observations by the attendant veterinarian on the state of health of the foals suckled by the mares are available. Of the remaining three, two foals died at birth; concerning the other no record was obtainable.

The records given in Table VII, taken in conjunction with the clinical history of the foals, are of sufficient importance to merit detailed consideration.

No. 72. This mare had had two foals, one in 1928 and one in 1929, both of which died when they were a day old. Her milk was analysed in 1929 on the day the foal was born and was found to contain 13·13 per cent. of fat. Another sample was obtained four days later and the fat percentage had then dropped to 6·62. This milk was obviously too rich in fat.

No. 78. The milk of this animal contained 12·47 per cent. of fat.

The foal lived for three days and died from jaundice. The milk was obtained the day the foal died.

No. 56. This was an abnormal colostrum milk containing 8·23 per cent. of fat. The foal died during delivery and consequently the effect of this milk could not be noted.

No. 9. The milk contained 7·88 per cent. of fat. The mare had a foal in 1928 and another in 1929. Both of them died from malnutrition when a week old. The composition of the milk, which was free from colostrum when sampled, is abnormal generally as the following figures show: total solids, 18·74 per cent.; protein, 7·60 per cent.; fat, 7·88 per cent.; sugar, 2·31 per cent.; ash, 0·95 per cent.

· *No.* 31. The milk contained 5·64 per cent. of fat. The mare had had two foals (1928 and 1929), both of which died when two days old.

No. 10 A. This mare was giving a large flow of milk and the foal had persistent diarrhoea. The milk was found to contain 5·30 per cent. of fat and presumably was too rich for the foal.

No. 9 A. The milk contained 4·40 per cent. of fat. The veterinary record of the mare, an aged hunter, is that she had had two foals, both of which appeared quite healthy at birth. The first (1928) died from malnutrition and the second (1929), at three weeks old, when the milk was sampled, was in an unthrifty condition, troubled with diarrhoea and not expected to live.

No. 22. This was colostrum containing 3·81 per cent. of fat. No record of the foal was available.

No. 13 A. The milk contained 3·6 per cent. of fat. The foal died as the result of an accident.

No. 15. This milk, containing 3·5 per cent. of fat, was obtained from a Clydesdale mare that had had two foals (1928, 1929), both of which throve well.

These records clearly support the contention that foals do not thrive on milk very rich in fat. In six out of ten instances when the mares were producing milk containing considerably more fat than the average, the foals failed to thrive; of the remaining four, two foals died from causes not associated with nutrition. Concerning one, no record was obtainable, while in another, where the milk contained 3·5 per cent. of fat, the mare was able to rear her foal satisfactorily.

On the other hand, during the course of this investigation evidence has been collected which shows that it is possible for foals to be well nourished when the fat is as high as 2·5 per cent., or, as in the instance of mare No. 15, even as high as 3·5 per cent.

Milk with low percentage of fat.

While the foregoing analyses give support to the common belief that milk very rich in fat does not agree with young foals, the investigation has also shown that foals can thrive on milk containing considerably less than the average amount of fat. Of the ten lowest percentages detailed in Table VII, with the exception of Nos. 84 and 2, there is no record of the foals doing badly, while on the other hand definite statements have been received from those sending the milk that the foals were doing well when the samples were taken. Of the two exceptions, No. 84, a Thoroughbred mare whose milk contained 0·29 per cent. of fat, the sample was sent by a veterinary surgeon with the statement that the mare had been "a bad mother for several years." Milk from No. 2 (a Shire mare) containing 0·30 per cent. of fat, was also sent by a veterinary surgeon with a request for information concerning the milk because the mare's previous foals had always done badly. In this case it is noted that particular care was taken to avoid the risk of obtaining "pressure" milk with a small amount of fat.

With these two exceptions there would appear to be evidence from the one hundred and forty-two samples tested that foals can be successfully reared when the dam's milk contains less than 1 per cent. of fat. The lowest percentages of fat which have been previously recorded are 0·12 per cent. by Fleischmann and 0·37 per cent. by Petersen and Höfker.

In view of the fact that the percentage of fat decreases with the increase of pressure within the udder, and that consequently the longer the animal is left unmilked the lower the percentage of fat, undue importance perhaps should not be attached to the low figures found in this investigation, notwithstanding the circumstance that every effort was made to obtain representative samples.

In order to determine the extent of the variability of the fat content when the milk is left in the udder for different periods, a Shetland pony mare with her foal a week old was obtained and housed in the College. The foal was kept from the mother for a lengthening time each morning, the udder being then emptied by hand and the fat percentage determined. Table VIII gives the results of the analyses and shows how quickly, in the mare, cessation from suckling (or hand milking) causes fat depression.

As previously said, the effects of stasis in the udder of cows is well known to lower the percentage of fat. This being so, it is reasonable to suppose that the same thing would occur in the mare under similar

conditions, and the figures given in Table VIII lend support to the sup-position. They furthermore suggest the probability that some—but not all—of the records of low fat percentages in mares may be due to this cause. Clearly, the old established practice of "milking out" the mare, when returning from work, before allowing the foal to suck is sound.

Table VIII. *Variations in fat in an individual mare. Pony* 87.

Hours separated from foal	Fat percentage	Hours separated from foal	Fat percentage
1¼	1·10	3	0·70
2	0·85	4	0·90
2	1·60	4	0·90
2	1·60	4	0·50
2	1·20	5	0·40
3	1·20	5	0·35

One may assume that the optimum amount of fat in milk is that which lies nearest to the average, provided that this represents the mean of a number of analyses sufficiently large to give a reliable figure; and provided, also, that the composition of the milk has not been modified by artificial selection in breeding, as has been done with dairy cows. The average percentage of fat in mare's milk, computed from the mean of former records and the mean of milks analysed in this investigation, is 1·44 per cent.

Presumably the farther the fat percentage is from the mean or "normal" in either direction, the less likely is the milk to meet the requirements of the young. We have no knowledge either of minimum fat requirements for the foal, or of the maximum amount that can be partaken of without causing digestive and metabolic disturbance.

When considering the nutritive value of milk for calves and lambs, definite live-weight gains can easily be ascertained and utilised as indices of the body's response to available nutriment; but in the case of foals this is not practicable, and judgment on the suitability of the milk can only be formed by studying the young animal's appearance, condition and general health.

In many of the cases recorded above, reports on the condition of the foal have been supplied by breeders and veterinary surgeons when the milk samples were sent to the laboratory, and from these one is led to conclude that foals can thrive well on milk containing as little as 0·2 per cent. of fat. On the other hand, milk containing 4 per cent. of fat (or possibly less) does not appear to give satisfactory results. Mares secreting milk rich in fat are not, therefore, to be regarded as ideal for breeding. The old established custom of giving preference to cow's milk

low in butter fat, or diluting the milk, when it is necessary to rear foals artificially, is therefore sound.

When studying the fat content of ewe's milk and its relation to growth, Ritzman(22) found great variation between individuals of the same breed and also in the milk of the same individual at different periods during lactation or during different lactation periods[1]. Ritzman analysed one hundred and fifty-eight samples of ewe's milk and found a range of 2·4 to 12·1 per cent. of fat and he expressed the view that fat is exceedingly variable in individual ewes, regardless of breed or age. The present investigation has disclosed the same to be true of mares, where, in one hundred and forty-two samples the range was from 0·09 to 13·13 per cent.

In discussing the results of his enquiry, Ritzman points out that where the entire yield of milk is taken by the young, a variation in the fat content can assume importance, inasmuch as it may be a determining factor in the rapid growth of the animal. He found that lambs consuming milk rich in fat did not thrive better than those getting milk poor in fat; in fact his figures seemed to indicate the reverse; but, as he points out, the *quantity* of milk yielded by the mother seems after all to be the determining factor.

In short, the main function of milk fat is to stimulate growth, and so long as that function is being fulfilled an excess of fat beyond the amount required for the purpose is not necessary but, on the contrary, may even be harmful.

Lactose.

The percentage of lactose in mare's milk is fairly constant, the average falling between 6 and 7 per cent. It is often considerably less than this in colostrum, and after the colostral period has passed, percentages higher or lower than the average are possible. Only four out of one hundred and four non-colostral samples analysed were found to contain 8 per cent. or over of lactose. Greater divergence from the average was found in samples that contained less than the normal.

Table IX gives the ten highest and the ten lowest percentages of sugar found in the samples examined.

There is no evidence in the history sheets of the foals that milk somewhat above the average in lactose is deleterious, provided that it is otherwise normal. On the contrary, two of the ten foals referred to in the table are reported "to be doing extremely well."

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[1] The same has also been noticed in cows.

Milk containing less than 4 per cent. of sugar, however, does not seem suitable, for the records show that the foals were unthrifty or had died of malnutrition.

It is interesting to observe that, among the ten lowest records of lactose, in only one instance (No. 9) was there an abnormally large fat content.

Table IX. *Abnormal lactose quantities (not including colostrum).*

Case no.	High (%)	Case no.	Low (%)
41	8·78	24 A	1·65
98	8·29	9	2·31
34	8·17	88	3·06
112	8·07	59	3·13
84	7·85	76	3·82
43	7·50	8	3·93
39	7·48	4 A	4·01
48	7·37	32	4·24
44	7·26	115	4·78
60	7·26	77	4·97

THE MILK OF AGED MARES.

It is well known that aged animals can successfully rear offspring, and analyses of the milk of old cows show that its composition is normal even up to the age of twenty-three years [23]. But search has failed to discover records of the yield and composition of the milk of aged brood mares.

As a contribution to the knowlege of this, and as a part of the investigation now being discussed, the milk of eighteen mares, aged ten years or more, has been examined. In no case was a sample taken during the colostral period. Table X shows the result of the analyses.

Table X.

No.	Age in years	Solids (%)	Protein (%)	Fat (%)	Sugar (%)	Ash (%)
4	10	11·11	3·88	0·31	6·17	0·75
29	10	9·26	1·99	0·25	6·73	0·29
102	10	11·26	2·60	1·89	6·27	0·50
19	12	11·47	2·80	1·68	6·42	0·57
48	13	12·97	2·76	2·28	7·37	0·56
85	13	10·83	2·65	1·58	6·01	0·59
92	13	13·02	4·62	2·32	5·44	0·64
45	14	11·43	3·23	0·67	6·99	0·54
15 A	14	8·95	2·91	0·40	5·19	0·45
93	16	10·77	2·53	2·40	5·42	0·42
70	17	12·97	6·26	2·01	1·91	0·79
98	18	12·05	1·52	1·82	8·29	0·42
20	18	12·26	2·42	3·35	6·09	0·40
55	19	8·64	2·08	0·35	5·78	0·43
27	20	11·68	2·26	1·99	7·01	0·42
124	24	12·12	3·29	2·14	6·09	0·60
95	Aged	11·81	3·10	2·21	5·88	0·62
9 A	Aged	13·93	3·23	4·40	5·86	0·44

A study of these figures reveals that aged mares, as in the case of aged cows, secrete milk that is not poor in quality; on the contrary, the milk has a tendency towards richness. Among these eighteen cases, however, there is a considerable degree of variation in all the constituents.

Colostrum.

Since it is possible to obtain samples at any desired time after parturition, analyses with cows have been made with some degree of completion. Unfortunately, examination of mare's colostrum offers much greater difficulty because of the reluctance on the part of the owner to consent to the taking of samples at definite intervals of time. An attempt was made to overcome the difficulty and procure material for analysis at definite periods by the purchase of an in-foal pony. Unhappily, the animal died before foaling.

Nevertheless, a certain amount of information has been gathered from the analysis of the milk of thirty-eight mares taken at various times up to and including the third day after parturition. The result of the analyses is given in Table XI.

These analyses show that mare's colostrum, so far as its composition has been studied, has the same characteristics as the colostral milk of other animals. It is much richer in mineral constituents, protein and total solids than normal milk and contains slightly less sugar. In regard to the percentage of fat, this enquiry has yielded results similar to those obtained in the analysis of cow's colostrum; fat may be normal or greater or less than the average. In four instances abnormally high percentages of fat were found: 13·13 per cent.; 12·47 per cent.; 8·23 per cent. and 5·64 per cent.; these have been discussed in connection with *abnormal milks*. One sample of colostrum, taken from the first milk drawn from a Thoroughbred mare (No. 57), was of a very viscid character and contained an unusually large quantity of protein (25 per cent.), the total solids being 28·88 per cent.

Judging from the samples examined, it seems clear that mare's milk is still definitely colostral in nature three days after parturition.

Other records of the analysis of mare's colostrum are scanty, but Camerer and Söldner[24] give the results of three analyses as follows:

	Protein (%)	Fat (%)	Sugar (%)	Ash (%)
1	7·82	2·64	4·39	0·63
2	8·37	1·77	4·24	0·67
3	6·10	2·33	4·49	0·64

Table XI. *The composition of mare's colostrum.*

No.	Hours after foaling	Solids (%)	Protein (%)	Fat (%)	Sugar (%)	Ash (%)
47	1st Milk	15·91	8·32	1·21	5·84	0·54
57	1st Milk	28·88	25·00	0·63	2·41	0·84
111	4	17·11	9·68	2·30	4·60	0·53
91	6	16·91	10·95	1·60	3·64	0·72
79	8	19·70	15·12	0·41	3·50	0·66
108	8	17·02	11·00	0·93	4·32	0·77
68	10	—	13·67	1·60	—	—
5	12	15·34	4·75	3·32	6·69	0·58
6	12	16·38	11·84	0·22	3·61	0·71
30	12	14·81	8·74	0·42	5·11	0·54
114	12	12·52	4·84	2·31	4·81	0·56
117	13	12·25	6·39	0·28	5·00	0·58
82	14	11·42	4·30	0·64	5·95	0·53
65	16	13·17	5·68	1·80	5·07	0·62
58	18	16·68	8·93	2·64	2·42	0·69
2	24	15·60	8·19	0·30	5·47	0·64
56	24	19·73	?	8·23	?	1·06
64	24	12·40	3·35	2·70	5·75	0·60
A 20	24	10·87	4·68	0·50	5·22	0·47
A 21	24	10·42	3·73	0·80	5·42	0·47
99	33	10·60	3·42	1·11	4·49	0·58
22	36	19·07	12·15	3·81	2·40	0·71
62	36	12·22	3·29	2·52	5·84	0·57
23	48	11·39	2·51	1·94	6·54	0·40
31	48	18·59	6·96	5·64	5·39	0·60
61	48	12·35	3·84	2·20	5·75	0·56
67	48	11·05	3·03	1·29	6·12	0·61
75	48	13·04	7·91	1·74	2·67	0·72
80	48	12·75	3·48	2·60	6·08	0·59
94	48	14·71	?	3·29	?	0·64
105	48	12·32	5·38	2·19	4·23	0·52
72	60	26·61	8·61	13·13	4·10	0·77
13	72	12·21	4·17	0·35	6·04	0·65
16	72	11·73	3·70	0·51	6·78	0·73
73	72	11·76	3·61	2·59	4·99	0·57
A 91	72	10·26	2·83	2·85	4·00	0·58
104	72	9·47	4·81	0·31	3·87	0·48
78	72	24·09	9·43	12·47	1·46	0·73

SUMMARY.

Samples of milk from one hundred and forty-two mares have been analysed for the purpose of determining the percentage of solids, protein, fat, lactose and ash.

Of this number, thirty-eight samples were taken during the period of colostrum secretion and one hundred and four were colostrum-free milks.

The average composition of the milk of British mares has been found to be in close agreement with that of continental mares. The only marked difference is in the percentage of ash.

The average percentage of ash in the milk of continental mares is

0·38 and in the milk of British mares 0·51. It has been found that in the lighter British breeds (Ponies and Thoroughbreds) the ash content is similar to that of the continental mares, whereas in the heavier breeds (Clydesdales and Shires) the percentage of ash is appreciably greater.

With the exception of the mineral content, there are no marked differences between the milks of the various British breeds.

Included in the samples examined were some from mares whose foals were not thriving; some of these milks were found to be abnormal.

The occurrence of oestrum in the mare commonly causes nutritional disturbance in the sucking foal.

Milk with a high percentage of fat is unsuitable for foals, and mares that secrete milk of this nature do not rear their foals satisfactorily. On the other hand, foals appear to thrive well if the milk contains very little fat.

The percentage of lactose in mare's milk is fairly constant. When divergence from the normal occurs, a slight excess does not appear to be harmful. Foals do not thrive when the milk contains much less lactose than is normally present.

Aged mares secrete milk that is normal or with a tendency towards richness.

The colostrum of mares has the same characteristics as the colostrum of cows.

Without the co-operation of breeders and veterinarians this enquiry could not have been made; this opportunity is therefore taken to express gratitude to those who kindly supplied milk samples for analysis.

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STUDIES ON SOIL REACTION.

VIII. THE INFLUENCE OF FERTILISERS AND LIME ON THE REPLACEABLE BASES OF A LIGHT ACID SOIL AFTER FIFTY YEARS OF CONTINUOUS CROPPING WITH BARLEY AND WHEAT. (AN EXAMINATION OF THE STACKYARD FIELD PLOTS, WOBURN EXPERIMENTAL STATION.)[1]

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(With Two Text-figures.)

THE completion in 1927 of a 50-year cycle of fertiliser experiments on continuous wheat and barley at the Woburn Experimental Station provided an excellent opportunity for examining the cumulative effects of fertilisers on the reaction and replaceable bases of a soil under extreme conditions. The experiments were laid down in 1876 by the Royal Agricultural Society to ascertain to what extent the striking success of fertilisers in maintaining the yields of continuous wheat or barley on a heavy clay loam soil at Rothamsted could be reproduced on a light sandy soil with much lower reserves of nutrient elements. The soil is derived from Lower Greensand material and had at the start little or no reserves of calcium carbonate. The mean rainfall is 660 mm. per annum. The plots were arranged systematically without replication in compact adjacent blocks in a field of more than usual uniformity. With a few minor modifications the winter wheat and the spring barley plots received similar fertiliser treatments, the principal comparison being no nitrogen *versus* ammonium salts *versus* sodium nitrate, each tested both alone and with the so-called "minerals" (superphosphate and potassium sulphate with sulphates of magnesium and sodium in addition for the first 30 years only). There were also comparisons of annual applications of nitrogenous fertilisers against double dressings in alternate years. Farmyard manure and rape cake were included to represent the organic manures then generally available.

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[1] This paper is based on data contained in J. K. Basu's "Thesis approved for the Degree of Doctor of Philosophy in the University of London."

The founders' anticipation that this light sandy soil might respond to fertiliser treatment in a different manner from the Rothamsted soil was abundantly realised. Although the yields were maintained on many of the plots for 30 years or so, ammonium salts which had proved so successful at Rothamsted that they were used in the majority of the plots as the standard form of nitrogen caused a striking reduction in yield of barley after about 15 years at Woburn and an almost complete failure after 20 years. A similar failure of the wheat followed later. This catastrophic loss of fertility aroused the greatest interest at the time, and after it had been found that the infertile plots were acid in reaction to litmus paper, halves of many of them were limed with very beneficial effects. These early results served to redirect attention to the importance of the old practice of liming which had fallen into decay as a result of the success of artificial fertilisers in soils such as the Rothamsted one, which contained reserves of chalk added by former generations of farmers. It is a striking feature of English agriculture that liming and chalking received much attention up to the first half of the nineteenth century, but comparatively little in the second half. The subject is scarcely mentioned in the writings of Lawes and Gilbert, though they were of course aware that the calcium carbonate in the Rothamsted soils was derived from the pits and dells with which the farm is riddled. The Woburn result focussed attention on the danger of this common neglect, and directly stimulated many of the researches which led to the modern study of soil reaction and replaceable bases.

The first liming of 1897 was soon found to be inadequate on many of the barley plots, and the liming was repeated at irregular intervals and in various dressings on both wheat and barley plots often with further subdivisions. In all cases part of the original unlimed plot was retained, and the crops failed almost invariably though the wheat often promised well during the winter.

The details of the fertiliser treatments and the amounts and times of liming are given in Tables I and II. From 1907 onwards the rates of fertiliser application were reduced. The nitrogen dressings were halved on most of the plots, the amount of potassium sulphate was greatly reduced, and the use of magnesium and sodium sulphate was discontinued. The crop yields fell off more rapidly after this change, apparently from nitrogen deficiency, and in the last few years were very low indeed. The necessity for more frequent and heavier liming on the barley plots shows that this crop is much more sensitive to acidity than wheat, as would be expected from its greater dependence on the surface soil.

After the completion of the 50-year cycle in the summer of 1926 the experiment was interrupted for fallowing. No manures were applied in the autumn of 1926 or in the years 1927 to 1930. After two years of fallow, crops were taken without manure in 1929 and 1930. In January, 1927, large soil samples were taken from each plot at six or more points; as there were no well-defined soil horizons, surface and subsoil samples were taken at the conventional depths 0–9 and 9–18 inches. Replaceable calcium and pH value by the quinhydrone method were determined on each of the samples; the other replaceable bases, the clay content, and the "degree of unsaturation" (or amount of "replaceable hydrogen") were determined on a selection of the more important plots. For convenience, all replaceable bases (or more correctly kations) are expressed as milligram equivalents per 100 gm. of air dry soil (1 mg. equiv. per cent. equals 0·028 CaO per cent.). The details of the analytical methods are given in the Appendix and the full results are set out for reference in Tables I, II, III and IV, together with the details of the manurings; condensed summaries of these results are given later   In the absence of adequate random replication it is impossible to estimate the statistical significance of the observed differences and, wherever possible, an attempt has therefore been made to average the whole of the relevant data on each of the questions considered, in order to minimise chance effects from soil and sampling irregularities. The duplication of the wheat and barley series and the circumstance that the arrangement of the plots is inverted in the two series go some way to eliminate general drifts in soil composition across the field. There is only one markedly erratic plot (Wheat 4), which has much more replaceable calcium than the adjoining plots or the corresponding one on barley and is situated on a pronounced mound and partly shaded by trees. Its subsoil contains much more clay than any other plot, and there can therefore be little objection to eliminating it from the averages. With this exception, the variation between similar treated plots is sufficiently close to inspire considerable confidence in conclusions drawn from the averages of several plots.

Notes to Tables I and II on earlier manuring—1877 *to* 1906.

(1) Till 1907 ammonium salts, equal weights of ammonium sulphate and ammonium chloride, were applied; since 1907 ammonium sulphate has been used. During the first 30 years on Plots 2, 3B, 5, 6, 8, 9, 11B (but not 3A) and on their corresponding limed portions the nitrogenous fertilisers and dung were applied at twice the rates given in the table.

(2) Minerals for the first 30 years were 3·5 cwt. superphosphate, 1·79 cwt. potassium sulphate, 0·90 cwt. sodium sulphate and 0·90 cwt. magnesium sulphate. Since 1906 3 cwt. superphosphate and 0·5 cwt. potassium sulphate were used.

Table I. Woburn barley plots 1927.

Plot no.	Fertilisers applied per acre per annum since 1907	Rate and time of liming (tons per acre)	Total lime added (tons per acre)	Total replaceable CaO in 0–18 in. (tons per acre)	Replaceable Ca mg. eq. % 0–9 in.	9–18 in.	pH 0–9 in.	9–18 in.	Replaceable Ca + H mg. eq. % 0–9 in.	9–18 in.	Clay content % 0–9 in.	9–18 in.	Mean yield of grain 1907–1926 (cwt. per acre)
Without nitrogen													
1	None	.	—	3·65	3·76	5·58	5·3	6·2	8·9	11·5	10·0	12·7	4·34
7	None	—	—	3·84	4·03	5·80	5·4	6·3	9·0	11·7	10·5	11·3	3·70
4A	Minerals	—	—	3·39	4·96	3·71	5·5	5·2	7·8	8·1	8·7	8·3	5·37
4B	Minerals + lime	1·0 in 1915	1·0	3·91	4·92	5·08	5·7	6·4	10·3	—	9·3	—	5·33*
Ammonium sulphate													
2A	0·184 cwt. N	—	—	2·08	1·16	4·16	4·4	5·5	8·0	9·5	9·3	9·7	0·46
5A	0·184 cwt. N + minerals	—	—	1·90	1·56	3·32	4·6	5·4	7·2	9·5	10·7	10·7	1·88
8A	0·368 cwt. N in alternate years + minerals	—	—	3·09	2·67	5·25	4·9	6·2	11·4	—	—	—	1·22†
8B	0·368 cwt. N in alternate years + minerals	—	—	3·08	2·08	5·80	4·5	6·1	11·3	—	—	—	0·59‡
Ammonium sulphate + lime													
2AA	N as 2A	0·25 in 1905, 1909, 1910, 1912; 0·5 in 1925	1·5	3·80	2·90	6·84	4·8	5·9	10·9	—	10·7	12·5	2·95
2B	N as 2A	2·0 in 1897, 1912	4·0	4·06	4·78	5·62	5·9	6·4	8·7	10·4	10·7	12·5	5·98
2BB	N as 2A	2·0 in 1897, 1905	4·0	4·53	5·01	6·60	5·7	6·5	12·4	—	—	—	5·50
5AA	N and minerals as 5A	1·0 in 1905, 1916	2·0	3·36	3·68	4·92	5·4	6·2	11·3	—	11·0	—	8·48
5B	N and minerals as 5A	2·0 in 1897, 1912	4·0	4·30	6·01	5·00	6·1	6·6	9·1	10·1	—	10·3	8·08
8AA	N and minerals as 8A	2·0 in 1897, 1912	4·0	3·98	4·92	5·28	5·9	6·6	12·2	—	—	—	9·17†
8BB	N and minerals as 8B	2·0 in 1897, 1912	4·0	4·35	5·42	5·72	6·1	6·5	10·9	—	—	—	6·28‡
Sodium nitrate													
3A	0·368 cwt. N	—	—	4·84	5·60	6·80	6·2	6·9	10·8	—	10·7	—	6·38
3B	0·184 cwt. N	—	—	4·23	4·95	5·88	5·8	5·9	10·2	10·7	11·0	11·7	5·34
6	0·184 cwt. N + minerals	—	—	4·26	5·46	5·46	6·1	6·3	9·6	10·7	10·0	12·3	8·61
9A	0·364 cwt. N in alternate years + minerals	—	—	4·67	5·31	6·64	5·8	6·9	11·4	—	—	—	10·74†
9B	0·364 cwt. N in alternate years + minerals	—	—	4·49	5·50	6·00	5·9	6·2	12·6	—	—	—	7·00‡
10A	0·184 cwt. N + superphosphate (after dung and no manure)	—	—	3·79	3·98	5·72	5·2	6·0	11·0	—	—	—	7·78
11A	0·184 cwt. N + potassium sulphate (after dung and no manure)	—	—	3·89	4·50	5·45	5·7	6·1	11·6	—	—	—	9·72
Sodium nitrate + lime													
3AA	N as 3A	2·0 in 1921	2·0	4·82	6·57	5·76	6·3	7·1	10·3	—	—	—	—
3BB	N as 3B	2·0 in 1921	2·0	4·84	6·84	5·56	6·4	6·6	10·3	—	—	—	—
Organic manures													
10B	0·184 cwt. N as rape dust	—	—	3·58	3·60	5·56	5·1	6·2	10·6	11·2	11·7	12·0	6·26
11B	0·74 cwt. N as farmyard manure	—	—	4·51	6·44	5·10	5·8	6·0	10·1	—	—	—	13·67

* 11 years. † N applied. † N omitted.

Table II. Woburn wheat plots 1927.

Plot no.	Fertilisers applied per acre per annum since 1907	Rate and time of liming (tons per acre)	Total lime added (tons per acre)	Total re-placeable CaO in 0–18 in. (tons per acre)	Replaceable Ca mg. eq. %		pH		Replaceable Ca+H mg. eq. %		Clay content %		Mean yield of grain 1907–1926 (cwt. per acre)
					0–9 in.	9–18 in.	0–9 in.	9–18 in.	0–9 in.	9–18 in.	0–9 in.	9–18 in.	
Without nitrogen													
1	None	—	—	3·74	4·76	4·82	5·2	5·8	9·6	9·6	11·3	10·0	4·46
7	None	—	—	3·34	4·08	4·48	5·0	5·8	9·7	10·1	9·5	10·5	4·07
4	Minerals	—	—	5·32	6·22	7·41	5·8	6·8	9·9	13·6	10·7	14·0	4·63
Ammonium sulphate													
2A	0·184 cwt. N	—	—	2·16	1·17	4·35	4·0	5·3	9·2	9·1	10·3	8·7	0·29
5A	0·184 cwt. N + minerals	—	—	2·52	1·82	4·64	4·1	5·9	9·8	10·8	10·5	11·7	6·96
8A	0·368 cwt. N in alternate years + minerals	—	—	2·02	1·24	3·94	4·4	5·5	11·9	—	—	—	2·42*
8B	0·368 cwt. N in alternate years + minerals	—	—	2·45	1·43	4·84	4·5	5·9	11·2	—	—	—	2·08†
Ammonium sulphate + lime													
2AA	N as 2A	0·25 in 1905, 1909, 1910, 1911	1·0	2·91	2·66	4·80	4·4	5·8	9·0	9·0	11·5	10·0	4·90
2B	N as 2A	2·0 in 1897	2·0	3·40	2·22	6·48	4·6	5·7	10·8	11·6	11·0	12·3	6·58
2BB	N as 2A	2·0 in 1897, 1905	4·0	4·29	5·40	5·39	5·5	6·2	10·6	—	10·0	—	6·13
5B	N and minerals as 5A	1·0 in 1905	1·0	3·74	4·29	5·28	5·5	5·9	9·4	10·5	11·3	10·5	9·70
8AA	N and minerals as 8A	0·5 in 1905, 1918	1·0	2·83	2·02	5·22	4·8	5·9	11·4	—	—	—	7·62*
8BB	N and minerals as 8B	0·5 in 1905, 1918	1·0	3·10	2·58	5·36	5·0	6·0	11·2	—	—	—	6·10†
Sodium nitrate													
3A	0·368 cwt. N	—	—	4·26	5·56	5·36	6·3	6·6	11·8	—	10·3	—	9·01
3B	0·184 cwt. N	—	—	4·07	5·16	5·25	5·7	6·2	9·3	10·5	9·5	10·0	7·79
6	0·184 cwt. N + minerals	—	—	4·19	5·24	5·48	6·0	6·2	9·6	9·7	10·0	10·5	9·15
9A	0·368 cwt. N in alternate years + minerals	—	—	4·23	5·16	5·68	6·2	6·7	10·7	—	—	—	9·20*
9B	0·368 cwt. N in alternate years + minerals	—	—	4·28	5·32	5·63	6·1	6·5	11·3	—	—	—	5·22†
10A	0·184 cwt. N + superphosphate (after dung and unmanured)	—	—	3·81	4·53	5·24	5·8	6·3	10·8	—	—	—	9·00
11A	0·184 cwt. N + potassium sulphate (after dung and unmanured)	—	—	4·07	4·61	5·81	5·9	6·3	10·7	—	—	—	7·90
Organic manures													
10B	0·184 cwt. N as rape dust	—	—	3·18	3·48	4·66	5·4	5·7	11·1	—	13·3	—	8·71
11B	0·74 cwt. N as farmyard manure	—	—	4·60	6·06	5·72	6·3	6·5	10·0	9·3	—	11·7	10·28

* N applied. † N omitted.

(3) Prior to 1907 Plots 10A, 11A, 10B had irregular treatments.

10A had farmyard manure (=0·74 cwt. N) 1877 to 1881, no manure 1882 to 1906 (except rape cake in 1889 as 10B).

11A had farmyard manure (=1·48 cwt. N) 1877 to 1881, no manure 1882 to 1906.

10B had farmyard manure (=0·74 cwt. N) 1877 to 1887, no manure 1888, rape cake (=0·36 cwt. N in 1889, and =0·74 cwt. N in 1889 to 1906).

Total amounts of N, P_2O_5, K_2O applied in tons per acre.

		1877–1906	1907–1926	Total 1877–1926
N (to Plots 2, 3B, 5, 6, 8, 9)	=	0·55	0·18	0·74
P_2O_5 (to Plots 4, 5, 6, 8, 9)	=	0·84	0·48	1·32
K_2O (to Plots 4, 5, 6, 8, 9)	=	1·34	0·25	1·59

Arrangement of plots.

Wheat					Barley		
1	2	3			3	2	1
			10	10			
4	5	6			6	5	4
			11	11			
7	8	9			9	8	7

Changes in replaceable calcium throughout 50 years.

Although a complete set of soil samples is available only for 1927 there are sufficient earlier samples to show that all of the plots have lost replaceable bases throughout the period of the experiment. Table III gives the replaceable calcium contents of these early samples and the corresponding ones in 1927. The average replaceable calcium content (in mg. equivalents per cent.) of the unmanured plots fell from 9·2 in 1876, to 7·2 in 1888, to 6·8 in 1898 and finally to 4·2 in 1927. The loss of calcium was much more rapid on plots receiving ammonium sulphate; after 50 years only 1·5 mg. eq. per cent. remained. Even this quantity is an overestimate, for it includes the water-soluble calcium which in these as in the other samples amounts to about 0·5 mg. eq. per cent. On plots with sodium nitrate or farmyard manure the loss of calcium was less than on plots without added nitrogen. These effects of nitrogenous fertilisers are well known, and a more detailed discussion of their magnitudes and probable mechanisms is given later in a survey of the whole of the relevant plots. The effect of minerals (essentially superphosphate and potassium sulphate) is not so clearly understood, and in spite of the limitation that the principal Woburn plots provide no test for the action of superphosphate and potassium salts separately, the fact that many of the major treatments occur both with and without minerals provides a good opportunity of testing some of the conflicting views commonly held on the action of superphosphate on the reaction and lime content of the soil.

Table III. *Replaceable calcium contents of Woburn barley and wheat plots in* 1876, 1889, *and* 1927 *in mg. eq. per* 100 *gm. of soil.*

In 1876 before applications of any manure:
Surface soil (0–9 inches) 8·32, 8·64, 9·90, 10·04; Mean 9·23.
Subsoil (9–18 inches) 5·92, 7·36; Mean 6·64.

Surface soils (0–9 inches)	1888 Wheat	1888 Barley	1898 Wheat	1898 Barley	1927 Wheat	1927 Barley
1. No manure	7·08	6·85	6·96	6·72	4·76	3·76
7. No manure	7·71	7·20	—	—	4·08	4·03
4. Minerals	7·16	5·53	6·01	5·13	6·22	4·96
2A. Ammonium salts	6·14	6·75	4·15	4·92	1·17	1·16
5A. Ammonium salts + minerals	5·47	5·44	4·36	3·65	1·82	1·56
3B. Sodium nitrate	7·93	7·36	—	6·58	5·16	4·96
6. Sodium nitrate + minerals	6·81	7·12	—	—	5·24	5·46
11B. Farmyard manure	8·41	7·67 ·	—	—	6·06	6·44

Average loss of replaceable Ca from 1888 *to* 1927 *(in mg. eq. per* 100 *gm. of surface soil).*

	Without minerals	With minerals
No nitrogen	3·05	0·75
Ammonium sulphate	5·28	3·76
Sodium nitrate	2·58	1·61
Average	3·57	2·04

The results in Table III show that the plots with minerals had less replaceable calcium in 1888 and 1898 and more in 1927 than the corresponding plots without minerals. There is, of course, some uncertainty in deductions drawn from the few early samples, but the consistency of the effect suggests that the discrepancy between the early and final samples may be a real one. In the first 30 years the plots with mineral manures received over 5 cwt. per acre per annum of sulphates of calcium, potassium, magnesium and sodium as compared with about 2 cwt. in the last 20 years. Before considering the effect of superphosphate in more detail it is necessary to ascertain whether the greater loss of calcium in the earlier years was accompanied by corresponding gains in other bases through direct kationic exchange.

THE REPLACEABLE CALCIUM, MAGNESIUM, SODIUM AND POTASSIUM.

Table IV gives the replaceable base contents for the barley plots without nitrogen in 1888 and 1898 and for all of the major wheat and barley plots in 1927. Even in the most acid soil the amount of replaceable calcium exceeds that of any other base and, on the average of the whole series of soils, calcium constitutes about three-quarters of the total replaceable bases, as is usual for soils of humid regions. Both potas-

sium and sodium are present only in small amounts, and no high accuracy is claimed for the analytical results for these elements.

Table IV. *Replaceable base contents in mg. eq. per 100 gm. of surface soil (0–9 inches) from Woburn barley and wheat plots in 1888, 1898, 1927.*

	Ca	Mg	K	Na	Mg + K + Na	Total bases	Saturation capacity
Barley 1888:							
1. Unmanured	6·85	1·06	0·25	0·47	1·78	8·63	—
4. Minerals	5·53	1·24	0·58	0·34	2·16	7·69	—
Barley 1898:							
1. Unmanured	6·72	0·81	0·23	0·38	1·42	8·14	—
4. Minerals	5·13	1·04	0·39	0·50	1·93	7·06	—
Average effect of minerals in 1888 and 1898 samples	−1·45	+0·20	+0·25	0·00	+0·45	−1·00	—
Barley 1927:							
1. Unmanured	3·76	0·67	0·17	0·26	1·10	4·86	9·9
7. Unmanured	4·03	0·68	0·15	0·23	1·06	5·09	10·0
4A. Minerals	4·96	0·78	0·35	0·27	1·40	6·36	9·1
2A. Ammonium sulphate	1·16	0·58	0·19	0·30	1·07	2·23	9·0
5A. Ammonium sulphate + minerals	1·56	1·13	0·27	0·35	1·75	3·31	8·9
2B. Ammonium sulphate + 4 tons lime	4·78	0·51	0·10	0·38	0·99	5·77	9·6
5B. Ammonium sulphate + minerals + 4 tons lime	6·01	1·02	0·25	0·22	1·49	7·50	10·5
3B. Sodium nitrate	4·95	0·58	0·16	0·34	1·08	6·03	11·2
6. Sodium nitrate + minerals	5·46	0·97	0·20	0·34	1·51	6·97	11·0
11B. Farmyard manure	6·44	1·44	0·30	0·58	2·32	8·76	12·4
Wheat soils 1927:							
1. Unmanured	4·76	0·63	0·11	0·28	1·02	5·78	10·5
7. Unmanured	4·08	0·69	0·12	0·32	1·13	5·21	10·7
4. Minerals	6·22	0·76	0·30	0·17	1·23	7·45	11·0
2A. Ammonium sulphate	1·17	0·55	0·14	0·31	1·00	2·17	10·1
5A. Ammonium sulphate + minerals	1·82	0·72	0·30	0·24	1·26	3·08	11·0
2B. Ammonium sulphate + 2 tons lime	2·22	0·38	0·08	0·22	0·68	2·90	10·6
5B. Ammonium sulphate + minerals + 1 ton lime	4·29	1·06	0·22	0·19	1·47	5·76	10·8
3B. Sodium nitrate	5·16	0·63	0·11	0·30	1·04	6·20	10·3
6. Sodium nitrate + minerals	5·24	1·09	0·14	0·50	1·73	6·97	11·2
11B. Farmyard manure	6·06	1·27	0·39	0·37	2·03	8·09	11·9
Average of 10 plots (1, 7, 2A, 2B, 3B) without minerals (1927 data):							
	3·61	0·59	0·13	0·29	1·01	4·61	10·2
Average of 8 plots (4A, 5A, 5B, 6) with minerals (1927 data):							
	4·45	0·94	0·25	0·29	1·48	5·93	10·4
Difference = average effect of minerals:							
	+0·84	+0·35	+0·12	0·00	+0·46	+1·31	+0·2

It is clear from the early results that where mineral manures were used the increase in replaceable magnesium and potassium is quite insufficient to balance the loss of calcium. The calcium must therefore

have been lost by exchange for hydrogen, *i.e.* by increased soil acidity. In the 1927 samples each of the replaceable bases is increased slightly by mineral manures.

Comparison of the early and the 1927 results shows that for all treatments other than farmyard manure there was a progressive loss of each of the replaceable bases throughout the experiment.

Table V. *Effect of nitrogenous fertilisers on replaceable bases.*

Means of barley (B.) and wheat (W.) plots each with and without minerals (1927 samples).
Replaceable bases in mg. eq. per 100 gm. soil.

Plots ...	No nitrogen B. 1, 7, 4 W. 1, 7, 4	Ammonium sulphate B. 2A, 5A W. 2A, 5A	Ammonium sulphate + lime B. 2B, 5B W. 2B, 5B	Sodium nitrate B. 3B, 6 W. 3B, 6
Ca	4·63	1·43	· 4·32	5·20
Mg	0·70	0·75	0·74	0·82
K	0·20	0·22	0·16	0·15
Na	0·25	0·30	0·25	0·37

In all cases there is more magnesium on the plots with minerals, even though none had been added for 20 years before the 1927 samples were taken. The increases (0·20 mg. eq. per cent. as the average of 1888 and 1898 and 0·35 in 1927) correspond to almost one-half of the total magnesium added since the commencement of the experiment in 1877, and thus provide evidence that replaceable magnesium is firmly held by the exchange complex. Although acidification by ammonium sulphate reduced the replaceable calcium to about one-quarter of that on corresponding sodium nitrate plots, its effect on the replaceable magnesium was so small as to be within the limits of the analytical determinations (Table V). These results are in complete accordance with the well-known difficulties of removing all the replaceable magnesium from soils by electrodialysis.

Although in every pair of comparable plots the replaceable potassium is higher where minerals have been added, the actual recovery is low, especially after 50 years (0·12 mg. eq. per cent. or about 5 per cent. of the amount added). There is no evidence that intense acidity has increased the loss of potassium. The lowest potassium contents are on less acid plots which carried relatively heavy crops without potassic manuring, *i.e.* with sodium nitrate and ammonium sulphate + lime. When the sodium nitrate plots were limed in 1921 there was a temporary increase in yield followed by a marked depression, which was maintained over the fallow period. This may be ascribed to a reduction in the availability of the small amount of potassium remaining.

There is no evidence of any appreciable increase in the replaceable

sodium on plots receiving sodium nitrate for 50 years or sodium sulphate for 30.

The results as a whole show that bases are held with increasing firmness in the order Na, K, Ca, Mg.

Table VI. *Effect of mineral manures on replaceable calcium:* 1927 *samples.*

O = no mineral manures; PK = with mineral manures.
Replaceable Ca in mg. eq. per 100 gm. soils.

	Without N		$(NH_4)_2SO_4$		$(NH_4)_2SO_4 + CaO$		$NaNO_3$	
	O	PK	O	PK	O	PK	O	PK
Plot numbers ...	1 and 7	4	2A	5A	2B	5B	3B	6
Barley series								
0–9 inches	3·9	5·0	1·2	1·6	4·8	6·0	5·0	5·5
9–18 ,,	5·7	3·7	4·2	3·3	5·6	5·0	5·9	5·5
0–18 ,,	4·8	4·3	2·7	2·4	5·2	5·5	5·4	5·5
Wheat series								
0–9 inches	4·4	6·2*	1·2	1·8	2·2	4·3†	5·2	5·2
9–18 ,,	4·7	7·4*	4·4	4·6	6·5	5·3†	5·3	5·5
0–18 ,,	4·5	6·9*	2·7	3·2	4·4	4·8†	5·2	5·4

* Wheat Plot 4 has abnormally high clay and replaceable base contents.
† Wheat Plot 5B received only 1 ton of lime per acre, whereas the corresponding plot without minerals (2B) received 2 tons of lime per acre.

Table VI gives the replaceable Ca contents of both surface soils and subsoils in 1927 for all of the pairs of comparable plots with and without minerals. The slight increase in replaceable Ca from mineral manures already mentioned is found in every one of eight pairs of comparable surface soils. It has already been shown that the other replaceable bases were also increased in the six pairs of plots tested, and the direct determinations of "unsaturation" or "replaceable hydrogen" confirmed the conclusion that the mineral manures increased the calcium by exchange by hydrogen, *i.e.* by reducing the acidity. In the subsoil samples the results are not consistent. In each of the barley plots the minerals reduced the replaceable calcium in the subsoil, but three of the wheat plots had more replaceable Ca in the subsoil as well as in the surface soil where mineral manures were used, the exception being a plot which received less lime than the corresponding plot without minerals. The barley results are consistent with the displacement of hydrogen ions from surface to subsoil by the addition of calcium sulphate in superphosphate, a result to be expected on leaching an acid soil with a dilute salt solution. No satisfactory explanation can be advanced for the apparent discrepancy between the barley and the wheat plots. Differences based on a few plots have, however, little significance, and by far the most important result of these determinations is the demonstration of

the inaccuracy of the view still held and taught in some quarters that superphosphate is an acid manure which uses up the lime of the soil. Although it is true that an effect in this direction was shown in the earlier samples and in the barley subsoils, the average effect after 50 years, when about 9 tons of superphosphate had been applied, was so slight that it must be regarded as quite insignificant. This result gains in importance when it is remembered that the Woburn soil loses lime so readily that plots with ammonium sulphate lost so much replaceable calcium in 20 years that the crops failed completely.

Unfortunately it is not possible to distinguish in the main plots between the effects of superphosphate and those of potassium and other sulphates. Since 1907 sodium nitrate has been used with superphosphate on Plot 10A and with potassium sulphate on Plot 11A. The replaceable calcium contents in 1927 show slightly more replaceable calcium in the potassium sulphate plots than in the superphosphate plots.

| | Wheat mg. eq. % | | Barley mg. eq. % | | Mean in tons CaO per acre |
	Surface	Subsoil	Surface	Subsoil	0–9 inches
10A sodium nitrate + superphosphate	4·53	5·24	3·98	5·72	3·80
11A sodium nitrate + potassium sulphate	4·61	5·81	4·50	5·45	3·98

Although the difference is small it supports the conclusion from the early samples that superphosphate in mineral manures tends at first to reduce the replaceable calcium slightly, even though the effect may disappear later.

The addition of monocalcium phosphate must involve the removal of some calcium from the exchange complex (or calcium carbonate where this is present) to form more basic and insoluble calcium phosphates which may be regarded as $Ca_3P_2O_8$, although there is some indication that only a still more basic salt is sufficiently stable to remain indefinitely in the soil. But the extra calcium removed from the complex is not lost from the soil and is probably still available to plants and capable of contributing to the buffer capacity of the soil. It may even return to the exchange complex in the course of time as the calcium phosphate reacts with colloidal iron oxide or alumina produced by the breaking down of the clay complex or by the normal processes of weathering. The reactions may be represented conventionally as:

(1) $CaH_4P_2O_8 + 2CaX \rightarrow Ca_3P_2O_8 + 2H_2X.$

(2) $Ca_3P_2O_8 + Al_2O_3 + 3H_2X \rightarrow 2AlPO_4 + 3CaX.$
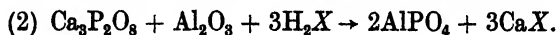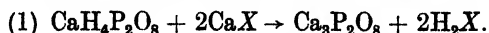
X represents the colloidal anions or exchange complex of the soil.

Such a sequence of reactions would account for the slight initial reduction and the subsequent increase in the replaceable calcium content of the Woburn soils treated with superphosphate.

The permanent effect of the gypsum is difficult to separate from the phosphate effect, but there is little doubt that its most important effect is an "activation of the soil acids," especially in dry periods in spring and early summer when the increased salt content displaces more hydrogen ions from the complex into the soil solution. If the old view of the acid action of superphosphate were founded on actual observations of more acute symptoms of soil acidity after the use of superphosphate, they may be explained by this temporary intensification of the acidity by neutral salt action or kationic exchange but not by a permanent loss of lime or acidification of the soil. The ultimate effect of addition of gypsum should be slightly to reduce the acidity and increase the replaceable calcium. All of these secondary effects are so small, however, that by comparison with the other fertilisers and for practical purposes it should be sufficient to regard superphosphate as without appreciable effect on soil reaction or lime status.

In the subsequent discussions in this paper the "minerals" and the "no minerals" plot will be averaged to increase the accuracy of the more important comparisons of nitrogenous fertilisers and the determination of the recovery of the added lime.

The effect of nitrogenous fertilisers.

The replaceable calcium contents in 1927 in all plots receiving the standard dressing of nitrogen as ammonium sulphate or sodium nitrate, together with the corresponding no nitrogen plots, are summarised in Table VII. For ease of comparison with the amounts of lime added, the replaceable calcium contents of the surface soil and the subsoil are converted into tons of CaO per acre to 18 inches, using for the soil density the mean value of 2790 tons per acre based on determinations about the middle of the 50-year cycle. Similarly treated plots show good agreement with the outstanding exception of the abnormally situated and heavier soil on Wheat Plot 4, which is omitted from the averages.

The mean values of replaceable lime in tons per acre are 2·4 for ammonium salts, 3·6 for no nitrogen and 4·3 for sodium nitrate. In terms of the total amount of nitrogen added throughout the experiment the effects are the extra loss of 0·80 mol. of CaO per mol. of $(NH_4)_2SO_4$ and the reduction of the loss by 0·48 mol. of CaO for the equivalent amount of nitrogen as sodium nitrate. These values do not agree with

Table VII. *Influence of form of nitrogen and amount of lime on replaceable calcium expressed as tons per acre, 0–18 inches, Woburn plots, 1927.*

All nitrogen dressings at the rate of 0·36 cwt. N per acre for first 30 years and 0·18 cwt. N per acre for next 20 years. Total N in 50 years = 0·74 tons per acre. B. = Barley, W. = Wheat: (*A*) = 8A, 8AA, 9A series; (*B*) = 8B, 8BB, 9B series.

Basal treatment		Ammonium salts with ·						No nitrogen No lime		Sodium nitrate No lime
		No lime	1 ton lime	2 tons lime	4 tons lime					
No minerals	B.	2·08	—	3·80*	4·06	4·53		3·65	3·84	4·23
	W.	2·16	2·91	3·40	4·29			3·74	3·34	4·07
With minerals	B.	1·90	—	3·36	4·30			3·39		4·26
	W.	2·52	3·74	—	—			(5·32)		4·19
With minerals annually and double N in alternate years	B. (*A*)	3·09	—	—	3·98			—		4·67
	B. (*B*)	3·08	—	—	4·35			—		4·49
	W. (*A*)	2·02	2·83	—	—			—		4·23
	W. (*B*)	2·45	3·10	—·	—			—		4·28
Mean		2·41	3·15	3·52†	4·25			3·59‡		4·30

* 1·5 tons of lime only added.
† mean 1·8 tons lime added.
‡ omitting exceptional Plot 4, wheat.

Effect on replaceable CaO.

	Ammonium salts to sodium nitrate	Ammonium salts to no nitrogen	Sodium nitrate to no nitrogen
Tons CaO for 0·74 ton N	− 1·89	− 1·18	+ 0·71
Mol. CaO for N equivalent to 1 mol. $(NH_4)_2SO_4$	− 1·28	− 0·80	+ 0·48

Amount of lime as applied in field required to raise replaceable Ca of ammonium plots to those of other plots (obtained by interpolation).

	Ammonium salts to sodium nitrate	Ammonium salts to no nitrogen	Difference
Tons CaO for 0·74 ton N	4·1	2·0	2·1
Mol. CaO for N equivalent to 1 mol. $(NH_4)_2SO_4$	2·8	1·4	1·4
Parts $CaCO_3$ per 100 parts $(NH_4)_2SO_4$ or equivalent N	210	· 104	106

the commonly accepted values for the "physiological acidity and alkalinity" of these fertilisers.

The effect of sodium nitrate can be explained simply by its "physiological alkalinity," *i.e.* by postulating that most of the nitrate is absorbed by the plant as nitric acid leaving an equivalent amount of sodium carbonate in the soil. Actual determinations of the nitrogen uptake on these plots are not available, but by combining the mean yields of grain and straw with estimates of their mean nitrogen percentages (1·5 and 0·35 per cent. respectively for the material as threshed and weighed) the mean recoveries of nitrogen for the plots given in Table VII are: for sodium nitrate 40 per cent. and for ammonium sulphate without lime

13 per cent. For sodium nitrate the value 40 per cent. (or 0·4 mol. of CaO per 2 mol. NaNO₃) agrees sufficiently well with the extra 0·48 mol. of CaO found in the soils that the conservation of calcium may be accounted for by assuming that all of the extra nitrogen is taken up as nitric acid. A similar assumption fails, however, for the ammonium plots where the loss relative to the no nitrogen plots is not that expected from full nitrification less nitrogen uptake (*i.e.* 2·0 − 0·13 = 1·87 mol. of CaO per mol. (NH₄)₂SO₄) but only 0·8 mol. At first sight this result appears to harmonise with Hall and Miller's [1] conclusions from studies on the loss of calcium carbonate from the Broadbalk plots at Rothamsted, viz. that 1 mol. of (NH₄)₂SO₄ increases the loss by 1 mol. of CaCO₃, but we have reasons to doubt the validity of this deduction from the Rothamsted data. The relatively low loss of lime in the acid Woburn plots depends on other factors, and is undoubtedly to be explained by the lower calcium bicarbonate concentration of the acid drainage waters percolating through soils with low replaceable calcium contents. Calcium is removed from the soil by drainage principally as Ca(HCO₃)₂, CaSO₄, and Ca(NO₃)₂, in amounts which fall off in this order. In the unlimed ammonium sulphate plots the replaceable calcium content is so low and the acidity of the soil solution so high that there will be much less bicarbonate for a given total carbonic acid concentration and, in addition, the actual carbon dioxide concentration of the soil air or water will be less on these plots which carry very small crops and receive little organic matter as crop residues. Independent evidence of small loss of lime as calcium bicarbonate on the more acid plots is provided by the limed ammonium sulphate plots. On the average about three-quarters of the first ton of added lime, about half of the second ton and only one-third of the next two tons are recovered as replaceable calcium. The loss of lime increases very rapidly as the replaceable calcium content increases, and very little of the added lime is lost from the more acid soils. The loss of calcium as bicarbonate on the unlimed ammonium sulphate plots must have been very small in the later years of the experiment.

If the acidifying effect of ammonium sulphate is estimated not by the actual loss of calcium but by the amount of lime required to raise the replaceable calcium contents of the ammonium sulphate plots to those of the no nitrogen and the sodium nitrate plots, much higher values are obtained. By interpolation it is found that 1·4 mol. of CaO are required per mol. of (NH₄)₂SO₄ to equal no nitrogen and a further 1·4 mol. to equal sodium nitrate. The comparison with no nitrogen has less significance than that of limed ammonium sulphate and sodium

nitrate, both of which give good crops. The difference between $(NH_4)_2SO_4$ and $2NaNO_3$ is equivalent to 2·8 mol. of CaO added in occasional limings, whereas the actual difference in replaceable Ca contents is only 1·3 mol. The discrepancy between these estimates and the theoretical value of 2 mol. CaO per mol. $(NH_4)_2SO_4$ brings out a practical aspect of the "physiological reaction" of fertilisers which is generally neglected in pot and laboratory experiments on this subject. Liming is rarely carried out annually; often indeed, as in these Woburn experiments, it is ignored until the untoward effects of the shortage compel attention. The resulting sporadic liming is necessarily wasteful, for it builds up temporarily relatively high replaceable contents and high rates of loss. Sodium nitrate provides a small though steady addition of base which is used efficiently.

In such extreme cases as the Woburn plots complications arise from the failure of the crop, the possible leaching out of other kations, such as iron, aluminium and even ammonium, and the decomposition of colloidal anions or clay. Agreement between theoretical and actual values of the effects of nitrogenous fertilisers cannot therefore be expected, but under normal farm conditions the effects should not differ seriously from those calculated under the only conditions in which exact treatment is possible, viz. when the size and composition of the crop is influenced by the amount but not by the form of nitrogenous fertilisers applied.

Following the early work of Meyer (2) it has been customary for continental workers to emphasise the "physiological acidity" of ammonium salts and to assume in the field a direct absorption of ammonia leaving a residue of sulphuric acid in the soil similar to that observed in water cultures, whereas British and American workers have emphasised the acidification by nitrification. It does not appear to have been generally realised that the apparent conflict between these views is purely formal and quite meaningless in view of our ignorance of the mechanism by which plants actually absorb and assimilate nitrogen. For constant absorption of nitrogen and calcium by the crop and equal locking up of nitrogen in soil organic matter, the loss of calcium from the soil can occur only as bicarbonate, nitrate, and sulphate, and under humid conditions the whole of the excess nitrogen will be washed out as nitrate whatever the form added or absorbed by plants and soil organisms. Unless a reserve of calcium carbonate is present or extra lime is added with the ammonium salts, the acidification by ammonium salts will lead ultimately, as at Woburn, to secondary effects which will modify these simple relationships and make it impossible to deduce the relative

effects of the nitrogenous fertilisers. Any general statement on this much discussed question must be restricted to the simplest case in which they are used under conditions in which the plants absorb the same amounts of calcium and nitrogen and the drainage water removes a constant amount of bicarbonate. Under such conditions the loss of calcium exceeds that for equivalent nitrogen added as nitrate by an amount equivalent to the excess of the anions over the kations in the added fertiliser, all added nitrogen being regarded as nitrate and all carbon and phosphates being ignored. The last qualification is required because carbon is eliminated from the soil largely as gas and phosphates remain in the soil. Table VIII gives the effects of a number of common nitrogenous fertilisers calculated on this basis.

Table VIII. *Theoretical effects of nitrogenous fertilisers in increasing the loss of lime from the soil.*

All values in mol. per 2N and relative to the effect of calcium or sodium nitrate.

No extra loss	Extra loss = 1 CaO (2 cwt. of lime per cwt. of nitrogen)	Extra loss = 2 CaO (4 cwt. of lime per cwt. of nitrogen)
$Ca(NO_3)_2$	NH_4NO_3	$(NH_4)_2SO_4$*
$2NaNO_3$	$(NH_4)_2CO_3$	$2NH_4Cl$
$CaCN_2$ (pure)	$CO(NH_2)_2$	
$NH_4NO_3 + CaCO_3$†	$2NH_4HCO_3$	
	$(NH_4)_2HPO_4$	
	$2NH_4H_2PO_4$	

* 1 cwt. ammonium sulphate increases loss by 0·82 cwt. lime or 1·47 cwt. calcium carbonate.   † Approximately "Nitrochalk" with 15·5 % N.

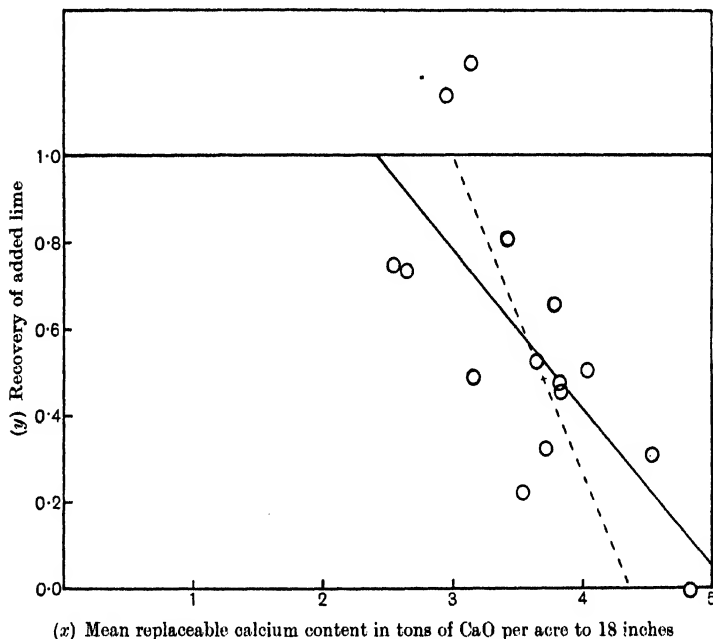### THE RECOVERY OF ADDED LIME.

The data already given for the major plots show that the loss of lime from the soil increases with the amount of lime actually present. To minimise irregularities in the soils and in the times of liming an attempt was made to summarise the results for all of the limed plots in the form of a regression equation relating the percentage recovery of the lime to the mean lime content of the top 18 inches in 1927. There were eight plots with one sub-division, two plots with two and one with three, making in all fifteen pairs of otherwise comparable plots.

The results are shown in Fig. 1 and Table IX.

The relationship is close enough to be regarded as highly significant. The regression lines suggest that for replaceable calcium contents of less than about 2·4 tons the whole of the added lime would be retained, whereas all of it would be lost under the conditions of these experiments if the replaceable lime content were more than 5·1 tons. It happens that the plot with the highest replaceable calcium (4·83 tons on the

Table IX. *Relation of the recovery of added lime to the lime content of soil.*

$x =$ mean replaceable lime of a pair of plots in tons per acre to 18 inches.
$y =$ recovery of added lime (*i.e.* ratio of difference in lime contents to amount of lime added).

Plot with more lime	W. 2AA	B. 5AA	B. 2AA	W. 5B	W. 2B	W. 8AA	B. 8AA	B. 4B	B. 8BB	W. 8BB	B. 5B	W. 2BB	B. 2B + 2BB	B. 3BB	B. 3AA
Plot with less lime	2A	5A	2A	5A	2AA	8A	8A	4A	8AA	8B	5AA	2B	2AA	3B	3A
x	2·54	2·64	2·94	3·13	3·16	3·42	3·54	3·65	3·72	3·78	3·83	3·84	4·05	4·54	4·83
y	0·75	0·73	1·15	1·22	0·49	0·81	0·22	0·52	0·32	0·05	0·47	0·45	0·50	0·31	−0·01

Correlation coefficient $r_{xy} = -0.71$ (for $P = 0.01$, $r = 0.64$).
Regression equation $y = 1.88 - 0.37x$; $y = 0$ for $x = 5.13$; $y = 1$ for $x = 2.40$.
$x = 4.36 - 1.38y$; $y = 0$ for $x = 4.36$; $y = 1$ for $x = 2.98$.

(*x*) Mean replaceable calcium content in tons of CaO per acre to 18 inches

Fig. 1. Lime recovery (*y*) and mean lime content (*x*) in 1927 from pairs of similarly manured plots. Regression lines (1) full line—$y = 1.88 - 0.37x$; (2) dotted line—$x = 4.36 - 1.38y$.

unlimed half, barley Plot 3A in 1927) was limed more recently than any other plot, but none of the lime could be detected 6 years later; other plots still contain appreciable fractions of the lime added 30 years ago.

As lime was applied at such irregular times, and more than once on most of the plots, it is not possible to connect the annual loss of lime with the amount present initially or added, but the closeness of the relationship between the recovery of lime and the mean lime content of the limed and unlimed plots shows that they are approaching equilibrium,

and that the total loss of lime is influenced much more by the amount present in the soil than by the interval since the last liming.

This dependence of the loss of lime on the lime content of the soil has important bearings on practice. Some systems of husbandry may be maintained almost indefinitely without liming on many moderately acid soils in humid districts, as, for example, in North Wales and Scotland. But when it is desired to introduce other more sensitive crops into the rotation a heavy initial liming must be given and followed up by smaller dressings at frequent intervals. In the original state the annual losses of lime were low and almost balanced by the normal supply of basic material from weathering and the return in crop residues of bases brought up by plant roots from the deep subsoil; but these processes are quite inadequate to balance the much greater annual losses from soil with the higher replaceable calcium contents required by more sensitive crops and these greater losses must be made good by periodic lining. Although a heavy initial dressing may be required to reclaim a very acid soil or to introduce more sensitive crops, it is recognised that it is better to maintain the higher level of lime by frequent small dressings than by occasional heavy ones. Regular dressings on moderately or slightly acid soils need not be as heavy as the annual loss of lime calculated from calcareous soils such as the Broadbalk field at Rothamsted. It has been found repeatedly that more lime is required in the field than in the laboratory to effect a given change in reaction, and it is doubtful whether the relationship between the two can be expressed by a constant factor as is sometimes claimed; the loss depends not on the amount added but on the amount present after the addition.

It is clear that there can be no general answer to the question "How long does a dressing of lime last?" In estimating the residual value of lime for compensation or other purposes a distinction should be made between heavy liming for reclamation and periodic liming for maintenance of a steady state. Lime used in the improvement of poor acid soils will retain much of its value for a long period of years and its value should be credited, whereas that used on more fertile soils once every four or five years can have no residual value at the end of the rotation.

Alkaline and "physiologically alkaline" fertilisers are often valued more highly than might be expected from laboratory measurements of their basicity and an example of the efficiency of sodium nitrate in conserving lime has already been given. Their special value lies partly in the provision of basic material at the time of greatest lime requirement by the crop and partly in the frequency of their use.

REPLACEABLE CALCIUM AND YIELDS OF BARLEY AND WHEAT.

The secondary effects of fertilisers on soil reaction and lime status proved to be the major factor in determining the yields in the Woburn experiments. This is indeed evident from the fact that it has been considered desirable to introduce so many sub-divisions for liming. Striking effects were again brought out in the 1929 and 1930 crops which were grown without manure after two years of complete fallow.

Table X. *Correlation coefficients between yields of grain and replaceable calcium contents of all the Woburn barley and wheat plots.*

All plots fallowed in 1927 and 1928 and without manure after 1926.
(Replaceable calcium contents in January 1927 samples.)

	Mean yield 1917 to 1926	Yield in 1929	Yield in 1930	No. of plots	For $P = 0.01$
Barley for CaO in 0–9 inches	0·62	0·76*	0·67	22	0·48
for CaO in 0–18 ,,	0·52	0·75*	0·62		
Wheat for CaO in 0–9 inches	0·71	0·70	0·72	26	0·54
for CaO in 0–18 ,,	0·67	0·65	0·72		

* Omitting the limed nitrate of soda plots, the correlation coefficients for 1929 are 0·90 for 0–9 inches and 0·84 for 0–18 inches.

Table X gives the correlation coefficients between the replaceable calcium contents of the soil (to 9 inches and to 18 inches), and the yields of grain for these two years and for the mean of the 10 years, 1917 to 1926. All of the correlation coefficients are positive and highly significant. In Fig. 2 the yields of all plots for the first crop after the two years fallow are plotted against the replaceable calcium contents of the surface soils in 1927. The data are divided to distinguish plots previously receiving until 1926 phosphorus and potassium from those without mineral manures. The farmyard manure plots are put in the first group and the rape cake plots in the second. In the barley plots the effect of the lime content is so marked that it masks effects from the presence or absence of residues of mineral manures except in two plots. Very low yields were obtained on the limed portions of the two plots with sodium nitrate alone for 50 years. There can be little doubt that this depression of yield by liming was caused by an intensification of the potassium deficiency resulting from high yields and one-sided manuring in the early years.

The general level of wheat yields were below those of barley, but they fell into two fairly well-defined groups each showing a good correlation with the replaceable calcium of the soil. Where the soil contained residues from mineral manures, the failure on the more acid soils was much less complete than on the plots without minerals or on the corresponding

barley plots. Wheat roots develop slowly during the winter, and by spring and early summer have reached the deeper and less acid subsoil before the acidity of the surface soil is intensified by the increased salt content of the soil water during dry spells. Spring-sown barley is much more dependent on the reaction and lime content of the surface soil.
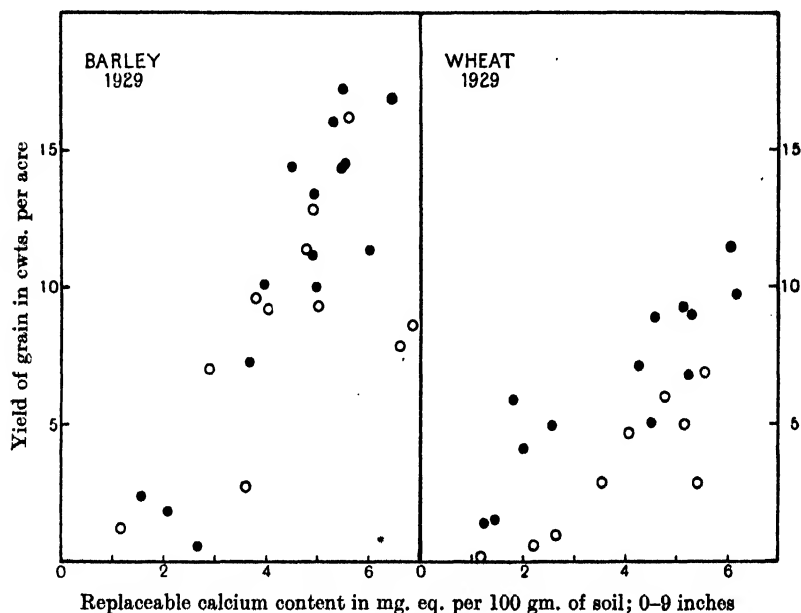
Fig. 2. Yields of barley and wheat unmanured after fallow for 2 years and continuous cropping for 50 years, without (rings) and with (dots) phosphatic and/or potassic fertilisers. Yields plotted against replaceable calcium contents in 1927 in mg. eq. per 100 gm. of soil to 9 inches.

The fate of the Woburn continuous barley and wheat plots demonstrates the paramount importance of adequate liming on light soils devoid of calcium carbonate. The experiments were planned to study a wide range of theoretical and practical problems on manuring, but all of the more direct effects of fertilisers on the plant were masked in later years by the indirect effects produced through their action on the lime reserves of the soil. At the outset 22 plots were considered adequate to cover the major fertiliser problems of light land; in the course of the experiment 17 additional plots were introduced to study the lime factor alone.

APPENDIX.

METHODS FOR THE DETERMINATION OF REPLACEABLE BASES
AND UNSATURATION.

Replaceable bases.

The following method was adopted:

25 gm. of soil passing the 1 mm. sieve were treated with 100 c.c. N NH$_4$Cl at 70° in a conical flask. After repeated thorough shakings the mixture was allowed to stand overnight and the supernatant liquid decanted through a filter. Extraction with 100 c.c. N NH$_4$Cl at 70° with frequent shaking during the first 15 minutes, followed by standing for 45 minutes and decantation, was repeated until the requisite volume of filtrate was obtained. Generally 500 c.c. were enough to remove all the replaceable bases. This method was found to be more rapid and more efficient than the more usual one in which the soil is transferred to a filter funnel after the first decantation and leached with cold solutions. Extraction with 0·5 N acetic acid gave similar results.

Calcium was precipitated as oxalate and determined volumetrically. The filtrate from the calcium oxalate was evaporated to dryness with nitric acid and ignited. Magnesium was determined as pyrophosphate and, after removing any sulphate by baryta followed by repeated precipitation with ammonia and ammonium carbonate, the alkali chlorides were weighed and potassium determined as perchlorate. pH values were determined by the quinhydrone method in 1 : 2·5 soil: water suspensions. The quinhydrone drift and error were very small.

DETERMINATION OF UNSATURATION OR "REPLACEABLE HYDROGEN."

In the present investigation, as in many analyses for advisory purposes, it is desirable to have some estimate of the "saturation capacity," or a direct determination of the "exchangeable hydrogen" or "unsaturation." Observed differences in replaceable calcium may be due to different amounts of exchange complex as well as to different degrees of saturation of the complex with calcium.

The choice of a method for estimating the degree of unsaturation is difficult, for none of the methods proposed for either "unsaturation" or "lime requirement" has any precise physico-chemical significance. They do not measure a definite amount of acid but are, at the best, convenient empirical measurements of the amount of base absorbed or acid liberated under arbitrarily defined conditions. In some simple

routine methods the conditions at equilibrium vary from soil to soil or with changes in the ratio of soil to solution. For soils under humid conditions with considerable leaching it is appropriate to select a method giving approximately 100 per cent. saturation to field soils which have retained considerable amounts of calcium carbonate for long periods. Since the usual method for determining replaceable calcium in calcareous soils involves extraction with N NaCl with some correction for the solubility of $CaCO_3$, we have used a method in which the soil is brought into approximate equilibrium with $CaCO_3$, N NaCl, and the air of the laboratory, as in replaceable calcium determinations on calcareous soils. The total calcium so extracted may, after suitable correction, be regarded as the sum of the replaceable calcium and hydrogen. The method has the additional merit that at no stage is the soil exposed to alkalinities high enough to decompose the exchange complex. A method based on these principles was proposed from this laboratory some years ago by H. J. Page and W. Williams[3] and subsequently studied and developed by P. E. Turner[4]. The method contains a serious source of error. The solubility of calcium carbonate was allowed for by Hissink's method of analysing the first and second litres of extract and taking the second as a measure of the solubility of $CaCO_3$ to be subtracted from the calcium content of the first litre. No notice was taken of the fact that suspensions of calcium carbonate and an acid soil in N NaCl necessarily contain calcium bicarbonate and have a pH value appreciably below 8·4. This is true even when an attempt is made to expedite the escape of carbon dioxide by allowing the system to stand in contact with air for several days, for as the carbon dioxide escapes the pH value of the suspension increases and more of the exchangeable hydrogen of the soil reacts with calcium carbonate. Hydrogen equivalent to the excess bicarbonate is thus counted twice, since the carbon dioxide liberated by the interaction of soil and $CaCO_3$ is also responsible for the dissolution of $CaCO_3$ to form $Ca(HCO_3)_2$. We have therefore subtracted the bicarbonate content, as determined by titration to a methyl red end-point in boiling solution from the total calcium content of the extract. After we had developed this method for "unsaturation" we found that Tiurin[5] had previously proposed a similar correction for exchangeable calcium determinations in calcareous soils.

    To reduce the amount of the correction and to accelerate the approach to equilibrium we have taken special precautions to remove carbon dioxide as rapidly as possible.

    10 gm. of soil (passing a 1 mm. sieve) and 2·5 gm. of calcium carbonate

(free from soluble salts) are made into a paste with a small amount of N NaCl in a conical flask. By suitable rotations the paste is spread out as a thin film on the sides of the flask and allowed to stand for a few hours until it is nearly dry. The soil is now shaken with 100 c.c. N NaCl at 70° and allowed to stand 45 minutes and exposed to the air with occasional stirring. The clear liquid is decanted through a filter into a 500 c.c. flask, and the extraction repeated in the same way until two or three successive 500 c.c. lots are obtained. The filtrate must be protected from contact with air to prevent the escape of carbon dioxide. 200 c.c. are titrated with 0·1 N acid and then used for the calcium determinations. In the light Woburn soils 1000 c.c. were sufficient to reach the end-point; in heavier soils 1500 c.c. were required.

Some typical results for extractions which were continued to 3000 c.c. are given in Table XI.

Table XI. *Woburn barley soils.*

Mg. eq. per 100 gm. of soil.

Number of 500 c.c. extract	Plot 2A			Plot 2B			Blank		
	Ca	HCO$_3$	Diff.	Ca	HCO$_3$	Diff.	Ca	HCO$_3$	Diff.
1	18·7	11·8	6·9	16·4	8·5	7·9	6·9	6·8	0·1
2	8·2	7·1	1·1	7·6	6·8	0·8	6·5	6·5	0·0
3	6·2	6·1	0·1	6·1	6·0	0·1	6·0	5·9	0·1
4	6·9	6·8	0·1	7·0	6·9	0·1	6·5	6·4	0·1
5	7·2	7·2	0·0	6·9	6·8	0·1	6·7	6·6	0·1
6	7·4	7·3	0·1	7·1	7·1	0·1	7·4	7·3	0·1
Total for 1st litre	26·9	—	8·0	24·0	—	8·7	13·4	—	0·1
Total for 2nd ,,	13·1	—	—	13·1	—	—	12·5	—	—
Difference	13·8			10·9			0·9		

	Replaceable Ca		Replaceable H		Replaceable Ca + H	
	2A	2B	2A	2B	2A	2B
Page-Williams method	1·16	4·78	12·6	6·2	13·8	10·9
By modified method	1·16	4·78	6·8	3·9	8·0	8·7

It will be noted that although the calcium contents of the blank fluctuated considerably, the true blank for calcium *minus* bicarbonate was consistently small and almost within the error of analysis. In the first 500 c.c. from the more acid soil (2A) the bicarbonate content was very high, and failure to allow for it introduced a 30 per cent. error into the saturation capacity even though the usual Hissink correction from the second litre were applied.

In the example given and in all of the data quoted in the paper the blank was so small that it could be neglected, but occasionally in a laboratory with several workers the amount of carbonic and other acids

in the air was sufficient to increase the blank considerably. It is desirable therefore to run a blank by the side of all determinations. Acid fumes and excessive carbon dioxide concentrations should be prevented, as the higher bicarbonate content of the resulting solutions retards the approach to equilibrium.

The sums of the replaceable Ca + H by the modified method agree much more closely than those by the Page-Williams method for the pair of limed and unlimed soils given in Table XI. For the Woburn soils tested the saturation capacity determined by this method was well correlated with the clay content and with the loss on ignition[1]. Acid soils from the Rothamsted Park Grass plots gave values for Ca + H only slightly higher than those on the arable land well supplied with calcium carbonate, whereas by the Page-Williams methods the Ca + H was very much higher on the acid soils through the failure to allow for calcium bicarbonate in the extracts.

The modified NaCl and $CaCO_3$ method was compared against a number of other methods on eight soils from the more important wheat plots. For economy of space the results are presented in Table XII by giving the mean for eight soils of the ratio of unsaturation by each of the methods to that by our modification together with the standard deviation of these ratios. The methods are arranged in order of decreasing agreement with our method in the relative values, *i.e.* in the order of increasing standard deviation of the ratios. A modification of Gehring and Wehrmann's method (6) and Kelley and Brown's (7) indirect method, in both of which the soil is treated with an excess of an alkaline earth hydroxide which is subsequently removed, gave results agreeing closely with those by our simpler method. The other methods tested have endpoints at pH 7·0 or less, and therefore give lower results for the unsaturation. The closest agreement with our method is obtained by the very simple determination of shaking the soil with calcium carbonate

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[1] For the 20 soils from the Woburn barley and wheat plots listed in Table IV, S, the sum of the replaceable kations (Ca, Mg, K, Na, H) is significantly related to C, the clay percentage and I, the percentage ignition loss by the equations:

$$S = 4·9 + 0·53 \ C; \quad S = 4·14 + 1·94 \ I; \quad S = 3·93 + 0·38 \ C + 0·78 \ I.$$

The first equation corresponds to an equivalent weight of clay of about 1890 if organic matter is ignored. Owing to changes in the relative amounts of replaceable bases caused by manuring, the sum of the Ca + H is not correlated with the clay in the surface soils but in 21 subsoils the following regression equation is significant:

$$S = 3·6 + 0·61 \ C,$$

and corresponds to an equivalent weight of clay of about 1640 neglecting the other bases and organic matter.

and sodium chloride and determining the total bicarbonate. The results are low though comparable with the Hutchinson-McLennan[8] calcium bicarbonate and Kappen[9] calcium acetate methods and, as in these methods, the pH at the end-point depends on the amounts of soil and solution taken.

Table XII.

	Mean ratio	Standard deviation of ratios as per cent. of mean ratio	Method	Procedure
1.	0·63	4·4	—	Measurement of HCO_3 produced when soil, $CaCO_3$, NaCl are left in contact for one week in a closed vessel
2.	0·99	7·4	Modified Gehring and Wehrmann ·	25 gm. treated with 100 c.c. saturated $Ca(OH)_2$ overnight. CO_2 passed through till colourless to phenolphthalein, followed by air until pink colour returns. NaCl added to give N NaCl; Ca determined by Hissink method
3.	1·02	9·2	Kelley and Brown	10 gm. treated with 100 c.c. 0·1 N $Ca(OH)_2$. Extracted with warm N NH_4Cl and excess removed with alcohol. NH_4 determined by distillation with MgO and replaceable bases subtracted
4.	0·61	10·4	—	10 gm. soil titrated electrometrically to pH 7 against 0·1 N alkali in presence of excess solid calcium acetate
5.	0·63	10·4	Kappen	20 gm. soil shaken with 100 c.c. N calcium acetate for 1 hour and filtrate titrated against 0·1 N alkali using phenol red
6.	0·55	10·9	Titration curve	20 gm. soil shaken with 100 c.c. of dilute $Ca(OH)_2$ solution for 3 hours, left overnight and pH measured by quinhydrone. Interpolation to pH 7·0
7.	0·72	12·5	Hutchinson— McLennan	10, 15, 20 gm. soil shaken with 0·02 N $Ca(HCO_3)_2$ for 4 hours. Filtrate titrated to methyl red end-point in boiling solution and results interpolated to a constant final bicarbonate concentration (10)
8.	0·60	17·0	CO_2 production from $CaCO_3$	Collection as in Hutchinson's method for $CaCO_3$ determinations of the CO_2 liberated on treating acid soil with $CaCO_3$ and N NaCl *in vacuo*

Summary.

1. At the completion of a 50-year cycle of continuous cropping with both wheat and barley on a light sandy soil at the Woburn Experimental Station, soil samples from all of the plots were analysed for replaceable bases.

2. The soil had little or no calcium carbonate originally and comparison with early soil samples showed that the unmanured plots lost about half of their replaceable calcium in the 50 years. The loss of calcium was much greater on plots with ammonium sulphate, and the crops failed completely in about 20 years. Plots with sodium nitrate or farmyard manure retained considerably more calcium than the unmanured plots.

3. Plots with mineral manures, essentially superphosphate and potassium sulphate, had less replaceable calcium in the earlier years but more in the surface soil at the end of the experiment than those without mineral manures. The effect of superphosphate was so slight that for practical purposes it may be regarded as without effect on the replaceable bases.

4. The final replaceable calcium was reduced at the rate of 0·8 mol. CaO per mol. of ammonium sulphate added throughout the experiment, and increased at the rate of 0·5 mol. for the equivalent amount of sodium nitrate. The relatively low value for the ammonium sulphate effect is due to the low base content of the very acid soils and the low calcium bicarbonate content of the water. To increase the replaceable calcium of the ammonium sulphate plots to that of the sodium nitrate plots required 2·8 mol. of CaO per mol. of ammonium sulphate when the lime was applied at intervals of about 10 years. A rule is proposed for calculating the effects of various nitrogenous fertilisers on the lime content of the soil.

5. Most of the added lime was recovered many years later when the original lime content was low, but added lime was rapidly lost by leaching from soils of high replaceable calcium content.

6. The yields of wheat and of barley in recent years were significantly correlated with the replaceable calcium contents of the soils.

7. A new method was devised determining the "degree of unsaturation" or "exchangeable hydrogen" of soils. A mixture of soil and calcium carbonate is extracted with N NaCl, and the difference between the calcium and the bicarbonate contents of the extract is taken as a measure of the replaceable calcium and hydrogen.

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EXPERIMENTAL ERRORS IN CHICKEN-REARING EXPERIMENTS.

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(With One Graph.)

EXPERIMENTS on the rearing of chickens have been carried out at the Agricultural Research Institute of Northern Ireland, Hillsborough, since 1928, and it was found necessary to examine the results statistically in order to determine their significance. Since the experiments carried out in 1929 and 1930 involved over 1500 birds in each year, it was thought that the general results of the statistical examination would be of sufficient interest to warrant their publication.

The experiments were designed to compare different rations and methods of management as regards their effect on the rate of growth of the chickens and on the subsequent laying powers, weight, etc., of the pullets. For each experiment, a lot of day-old White Wyandotte chicks, all hatched at the same time, was divided at random into the necessary number of groups. The groups were then placed under hovers in different houses, but the houses, methods of feeding, etc., were identical for all groups, except for those conditions which were under trial. The mash part of the ration was fed dry in every case, and except for particular groups, the chicks also received scratch grain, grit and water in the usual way, and were allowed out on grass runs when the weather permitted. The groups were weighed several birds at a time, at weekly intervals for the first few weeks, and later at fortnightly intervals. When the cockerels were large enough for sale and could be distinguished with certainty from the pullets, as was usually the case when they were about 13 weeks old, the birds were weighed individually. The sexes were usually separated at this time, the cockerels being sold and the pullets carried on for laying experiments.

The individual weights were recorded in grams and taken to the nearest 25 gm. They provide the basis for estimates of the significance of differences between groups and are the figures used for this paper. Since the weight of a day-old chick is only 40 to 50 gm., the live weight

figures can be taken as recorded as measures of the growth of the groups, and it is not necessary to deduct the weights at the beginning of the experiments. In each group the average weight per bird and the standard deviation of the weights were calculated for the group as a whole and for the cocks and pullets separately. The usual formula for the standard deviation was used, namely,

$$\text{Standard deviation} = \sqrt{\frac{Sx^2}{n-1}},$$

in which Sx^2 represents the sum of the squares of the deviations of the weights from the mean weight and n represents the number of birds. The standard deviations were converted to coefficients of variation, *i.e.*

Table I. 1929 *experiments; numbers of birds, mean weights and standard deviations.*

Experimental group	Both sexes			Cocks			Pullets		
	No.	Mean weight (gm.)	Standard deviation, % of mean	No.	Mean weight (gm.)	Standard deviation, % of mean	No.	Mean weight (gm.)	Standard deviation, % of mean
A	53	1354	18·64	24	1535	15·01	29	1204	12·66
B	68	1199	16·57	30	1333	13·39	38	1093	13·14
C	52	1316	13·09	28	1418	11·53	24	1198	7·12
D	53	1104	15·38	26	1195	12·00	27	1017	14·50
E	37	1358	15·96	15	1530	12·51	22	1241	11·59
F	35	1346	11·42	18	1440	8·40	17	1246	9·48
G	36	1452	13·65	23	1557	9·85	13	1267	9·20
H	33	1372	16·26	12	1617	9·90	21	1232	7·76
J	99	1497	16·57	57	1657	11·59	42	1280	8·81
K	97	1484	15·67	50	1654	10·55	47	1303	9·61
L	100	1297	15·91	42	1439	13·84	58	1194	11·76
M	59	1381	14·82	28	1493	12·24	31	1279	13·17
N	57	1291	16·43	22	1428	14·69	35	1205	13·67
O	53	1395	17·44	27	1559	12·59	26	1225	12·59
P	60	1336	18·09	31	1466	15·61	29	1197	14·09
Q	59	1382	15·90	35	1513	11·97	24	1192	8·17
R	65	1405	16·48	32	1583	11·39	33	1233	9·40
S	59	1377	16·80	29	1491	16·89	30	1268	11·30
T	62	1289	14·12	34	1357	13·81	28	1207	11·49
U 1	52	688	26·31	30	686	28·09	22	690	24·35
2	52	1379	24·33	30	1411	26·82	22	1336	20·15
V	57	1475	15·47	34	1583	14·34	23	1317	8·06
W 1	56	1020	19·18	30	1076	16·90	26	956	20·38
2	56	1421	17·87	30	1531	15·67	26	1293	16·13
X 1	63	954	26·62	30	1119	16·66	33	804	26·26
2	63	1309	22·73	30	1525	11·21	33	1113	22·32
Y	72	1459	14·18	32	1624	8·62	40	1328	11·22
Z (a)	64	1500	17·71	33	1691	11·64	31	1298	12·07
Z (b)	65	1445	17·39	33	1612	13·91	32	1273	10·56

Table II. 1930 *experiments; numbers of birds, mean weights,*
and standard deviations.

	Both sexes			Cocks			Pullets		
Experimental group	No.	Mean weight (gm.)	Standard deviation, % of mean	No.	Mean weight (gm.)	Standard deviation, % of mean	No.	Mean weight (gm.)	Standard deviation, % of mean
A	58	996	24·84	33	980	30·78	25	1017	14·93
D	69	1243	16·92	31	1360	17·27	38	1147	10·93
E	68	1310	20·31	33	1460	14·71	35	1168	19·79
F	105	1331	19·26	56	1467	15·26	49	1177	16·82
G	93	1279	17·56	50	1357	17·34	43	1188	14·65
H	103	1326	15·67	53	1422	15·27	50	1225	11·35
J	69	1394	17·45	39	1492	17·01	30	1266	12·32
K	69	1153	17·65	33	1270	15·71	36	1044	13·15
L	62	945	19·62	22	1017	20·00	40	906	18·13
M	63	1208	17·11	29	1299	18·15	34	1130	12·37
N	70	1244	17·76	43	1353	14·05	27	1070	13·31
O 1	71	588	27·62	35	602	30·72	36	574	24·08
2	69	1062	26·46	36	1117	28·98	33	1002	21·40
3	69	1511	22·54	33	1627	24·05	36	1405	17·59
P 1	90	1038	23·75	39	1161	22·06	51	943	20·47
2	90	1347	22·98	41	1497	24·52	49	1221	14·14
R	109	1454	20·28	59	1558	20·95	50	1331	14·49
S	95	1254	20·35	45	1388	20·40	50	1133	12·90
U	95	1520	17·46	50	1658	15·82	45	1367	12·26
V	106	1498	19·74	61	1605	19·38	45	1354	14·76
W	44	1417	24·97	18	1507	27·37	26	1355	22·12
X	102	1491	18·53	53	1676	13·58	49	1293	12·86

were expressed as percentages of the mean weights, so that the variabilities of groups can be compared although they differ in mean weight. These figures, together with the numbers of birds in each group, are tabulated for the 1929 and 1930 experiments in Tables I and II respectively. These tables, however, cannot be interpreted satisfactorily apart from the history and treatment of the birds, and data are given in Tables III and IV as to the time of hatching, age when weighed, and experimental treatment of the different groups. The treatments can only be summarised briefly here, and it is only necessary to include them because they have considerable influence on the variability of the weights. The results of the experiments are, or will be, published elsewhere.

It will be seen at once that the standard deviations vary considerably from group to group, and, while a large part of the variations can be attributed to chance in the selection of birds, many of the larger figures can only be caused by special conditions. In some cases, such as Group U in 1929 and Groups A, O, P and W in 1930, the treatments gave very poor rates of growth, and this may be accounted the cause of the high variability. Groups O and P in 1930 were also affected by

an outbreak of coccidiosis, which attacked Groups R and S at the same time, and will account for the rather high standard deviations in these two groups. Disease also accounts for the high deviations of the later hatched groups in 1929, since Groups U, V, W, X, Y, Z (a) and Z (b) suffered from worms.

Table III. 1929 *experiments; dates of hatching,*
ages when weighed, and notes.

Experimental group	Date of hatching	Age when weighed individually	Notes on experimental treatment
A	Jan. 19	13 weeks 5 days	Cereals, separated milk
B	,, 19	Do.	Cereals, minerals, soya meal at different rates
C	,, 27	12 weeks 5 days	Cereals, separated milk
D	,, 27	Do.	Cereals, minerals, soya meal at different rates
E	Feb. 6	13 weeks 1 day	Cereals, separated milk
F	,, 6	Do.	Cereals, minerals, soya meal
G	,, 16	12 weeks 5 days	Cereals, separated milk
H	,, 16	Do.	Cereals, separated milk, bone flour, soya meal
J	Mar. 3	13 weeks 4 days	Cereals, separated milk
K	,, 3	Do.	Special mixed ration
L	,, 3	Do.	Modification of mixed ration
M	,, 13	13 weeks 1 day	Cereals, separated milk, oystershell
N	,, 13	Do.	Cereals, separated milk, lime-free gravel
O	,, 13	Do.	Cereals, separated milk, no grit or shell
P	,, 29	13 weeks	Mixed ration, as *K*
Q	,, 29	Do.	Modification of mixed ration
R	,, 29	Do.	Do.
S	,, 29	Do.	Do.
T	,, 29	Do.	Do.
U 1	Apr. 8	13 weeks 3 days	Cereals, water
2	,, 8	21 weeks 3 days	—
V	,, 8	13 weeks 3 days	Cereals, separated milk
W 1	,, 8	Do.	Cereals, water, 4 per cent. minerals
2	,, 8	16 weeks 2 days	—
X 1	,, 8	13 weeks 3 days	Cereals, water, 6 per cent. minerals
2	,, 8	16 weeks 2 days	—
Y	,, 25	14 weeks	Cereals, separated
Z (a)	,, 25	Do.	Cereals, minerals, soya meal at different rates
Z (b)	,, 25	Do.	Cereals, minerals, soya meal at different rates

As a rule, the cockerels are rather more variable in weight than the pullets in the same group, but in Groups D, F, M, W, X and Y of 1929 and Groups E and F of 1930 the pullets vary more than the cockerels. It is difficult, however, to account for the variability of the pullets in these groups, since they have not made especially poor growth in proportion to that made by the cockerels in the same groups.

The data available do not give any information as to whether early hatched birds give more uniform gains than do those hatched later, since any effect of time of hatching is masked by other factors. Nor is

Table IV. 1930 *experiments; dates of hatching,*
ages when weighed and notes.

Experimental group	Date of hatching	Age when weighed individually	Notes on experimental treatment
A	Jan. 11	13 weeks 4 days	Cereals, separated milk; kept under Vita glass
D	,, 11	Do.	Cereals, separated milk; kept under ordinary glass, cod-liver oil in mash
E	,, 11	Do.	Cereals, separated milk; ordinary glass, allowed out and windows raised
F	,, 21	12 weeks 6 days	Separated milk, cereals and grain separately
G	,, 21	Do.	Separated milk, cereals, no grain
H	,, 21	Do.	Separated milk, cereals and grain mixed
J	Feb. 5	13 weeks	Cereals, separated milk
K	,, 5	Do.	Cereals, ½ separated milk ½ water
L	,, 5	Do.	Cereals, ¼ separated milk ¾ water
M	,, 5	Do.	Cereals, ¼ separated milk ¼ water, minerals, soya meal
N	,, 5	Do.	Cereals, separated milk 5 weeks; later minerals, soya meal
O 1	,, 15	20 weeks 3 days	Cereals, water
2	,, 15	26 weeks 4 days	—
3	,, 15	30 weeks 6 days	—
P 1	,, 15	20 weeks 3 days	Cereals, water, minerals
2	,, 15	22 weeks 4 days	—
R	,, 15	20 weeks 3 days	Cereals, separated milk
S	,, 15	20 weeks 3 days	Cereals, water, minerals, soya meal
U	Mar. 2	15 weeks 2 days	Cereals, soya meal, minerals
V	,, 2	15 weeks 2 days	As U, minerals without bone flour
W	,, 2	21 weeks 4 days	As U, minerals without chlorides
X	,, 2	15 weeks 2 days	As U, minerals without iron, sulphur and iodide

there much evidence as to whether the standard deviations are reduced by a longer growing period, although in the case of the five groups which were weighed individually more than once, the standard deviations of the later weighings are, in every case but one, the lower. It may be noted, however, that two American workers[1] have published results of an experiment in which the chickens were weighed individually at weekly intervals. Some of the figures from this experiment are given in Table V since they provide useful comparisons with the experiments under discussion.

Table V. *Data of experiment by Titus and Jull.*

	Cocks		Pullets	
	No milk	Milk	No milk	Milk
Coefficients of variation at time of hatching	9·48	7·91	8·87	10·36
Maximum coefficients	33·83 (7th week)	20·90 (3rd week)	26·05 (8th week)	23·61 (4th week)
Coefficients at 13th week	23·42	10·71	20·23	13·82
Mean weights at 13th week (gm.)	1052	1474	845	1135
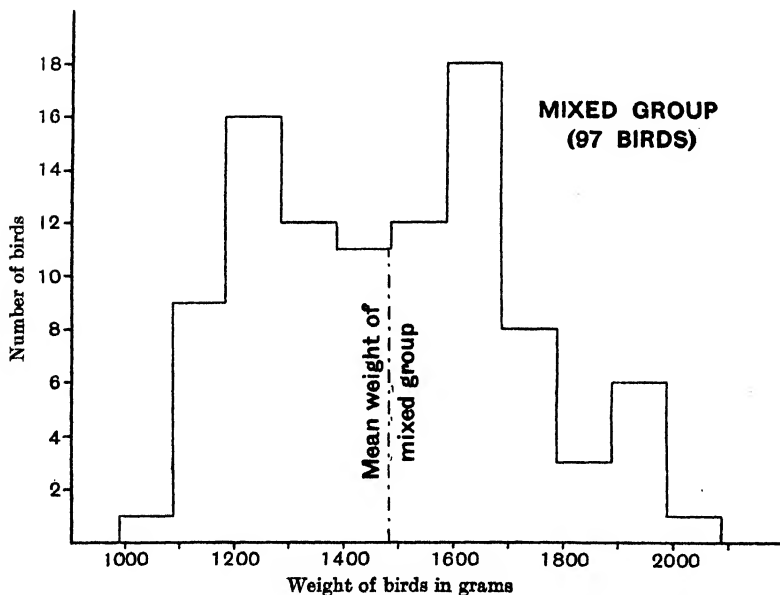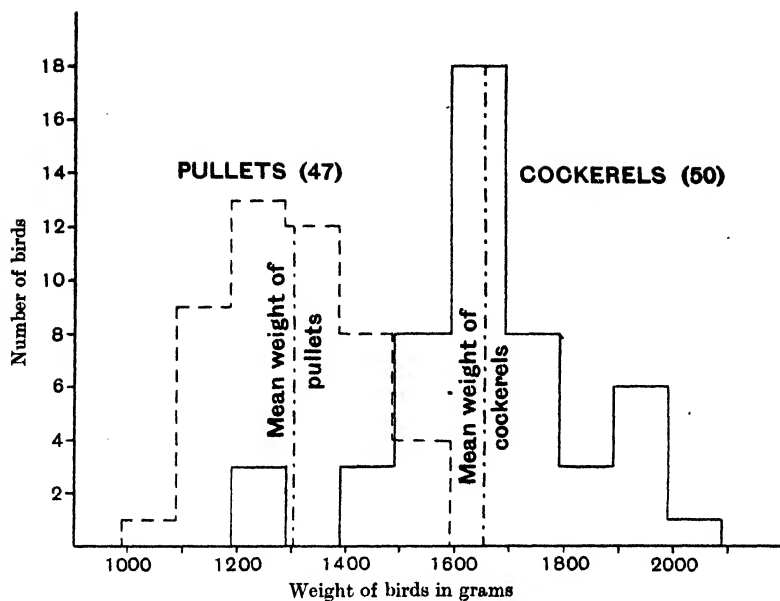
Graph. Frequency distributions of weights in group K, 1929, for cockerels and pullets and for the mixed group.

The coefficients of variation, as shown in the table, increased rapidly for the first weeks of growth and afterwards decreased slowly. In the groups getting skim milk, which made much the better growth, the increase in the coefficients lasted only 3 or 4 weeks, as against 7 or 8 weeks for the groups not getting skim milk. It may also be noted that the groups making the poorer growth have the greater coefficients, which agrees with the effects mentioned above in the Hillsborough experiments.

The difference between the average weights of cockerels and pullets makes the means of mixed groups of both sexes of doubtful reliability. The average weight of the pullets is generally about 80 to 85 per cent. of the weight of the cockerels, although in these experiments it is as low as 73 per cent. and in one case, Group A in 1930, is actually slightly greater. In this group, however, the high average weight of the pullets may be put down to high mortality amongst the worst pullets rather than to a high average rate of growth for all the pullets. From these experiments it is evidently possible for there to be considerable inequalities in the numbers of each sex in any group, although these inequalities must have arisen almost entirely by chance during the division of the day-olds into the experimental groups. Comparisons of mixed groups are thus made unnecessarily rough. Moreover, some treatments favour one sex more than another, and this effect will be masked unless cockerels and pullets are averaged separately.

The standard deviations of mixed groups also must be used with caution, since the frequency distribution of the weights in such groups is generally bimodal. The means of such groups may, of course, be more or less normally distributed, so that the standard deviations may without great inaccuracy be used to calculate the standard errors of the means and the significance of their differences. But it must be remembered that the standard deviations cannot be applied in the usual way to measure the variability in the groups and that their size will be very largely affected by the differences between mean weights of cockerels and pullets. A graph is given of the distribution in Group K, 1929, as an example of the type of distribution usually found. Apart from irregularities which may be attributed merely to the small numbers of birds involved, it will be seen that the separate graphs for cockerels and pullets have definite single modes, but that the graph for the mixed group is bimodal. The graphs for the separate sexes do not represent a sufficient number of birds for a test of strict normality of distribution to be made.

From a general consideration of the standard deviations shown in Tables I and II, and from comparisons with the experiment mentioned above and with other American figures, it may be concluded that, where the experimental treatment and other conditions affecting growth have been satisfactory, the standard deviations of the live weights, the sexes being taken separately, should not be more than about 15 per cent. of the mean weight for the group when the birds are about 13 weeks old, or big enough for the sexes to be distinguished. Very few of the groups which made good growth show standard deviations greater than this and it would appear to be a fairly safe figure to assume when considering questions of significance of results and of numbers of birds required to demonstrate differences of given sizes. Table V has been constructed for these purposes, assuming standard deviations of 15 per cent., 17·5 per cent. and 20 per cent. It shows firstly, for the assumed standard deviations and for given numbers of birds, the smallest differences between mean weights of groups, expressed as percentages of the mean for both groups, which can be considered significant. The customary criterion of significance is used, namely, that a difference shall be at least twice its own standard error. The smallest significant percentage differences are applied to a range of mean weights corresponding roughly to those found in the experiments, giving smallest significant differences in grams for the given mean weights. The table can thus be used for rough determinations of the significance of differences between groups. Where the chickens are reasonably even in growth and a standard deviation of 15 per cent. can be assumed, the differences to be significant must be at least as great as those shown in the table for the size and number of birds in question. Any roughness due to taking numbers and weights in round figures will cause errors amounting to only a few grams, which will hardly vitiate the results, since the method itself is in any case quite arbitrary. Greater inaccuracies in applying the table are likely to arise from the assumption of a standard deviation without calculating it, and it is suggested that the table can be used only for rough indications of the reliability of results if these are required before final results are available. For instance, indications may be required before individual weighings have been made. As 15 per cent. may be too low a figure to assume when the birds have not done well, the table includes standard deviations of 17·5 per cent. and 20 per cent. to meet such cases.

Table VI. *Smallest significant differences in chicken weights, for given standard deviations, numbers of birds, and mean weights of birds, for experiments commencing with day-old chicks.*

No. of birds in groups	Smallest significant differences, % of mean	Smallest significant differences in grams, when mean weights in grams are as under					
		1100	1200	1300	1400	1500	1600
Standard deviation 15 per cent. of mean weight							
10	13·4	148	161	174	188	201	215
20	9·5	104	114	123	133	142	152
30	7·7	85	93	101	108	116	124
40	6·7	74	80	87	94	101	107
50	6·0	66	72	78	84	90	96
60	5·5	60	66	71	77	82	88
70	5·1	56	61	66	71	76	81
80	4·7	52	57	62	66	71	76
90	4·5	49	54	58	63	67	72
100	4·2	47	51	55	59	64	68
Standard deviation 17·5 per cent. of mean weight							
10	15·7	172	188.	203	219	235	250
20	11·1	122	133	144	155	166	177
30	9·0	99	108	117	127	136	145
40	7·8	86	94	102	110	117	125
50	7·0	77	84	91	98	105	112
60	6·4	70	77	83	89	96	102
70	5·9	65	71	77	83	89	95
80	5·5	61	66	72	77	83	89
90	5·2	57	63	68	73	78	83
100	4·9	54	59	64	69	74	79
Standard deviation 20 per cent. of mean weight							
10	17·9	197	215	233	250	268	286
20	12·6	139	152	164	177	190	202
30	10·3	114	124	134	145	155	165
40	8·9	98	107	116	125	134	143
50	8·0	88	96	104	112	120	128
60	7·3	80	88	95	102	110	117
70	6·8	74	81	88	95	101	108
80	6·3	70	76	82	89	95	101
90	6·0	66	72	78	83	89	95
100	5·7	62	68	74	79	85	91

SUMMARY.

The results of a number of experiments carried out at Hillsborough on the growth of chickens, covering two seasons and involving 45 groups of 30 to 100 chickens, are discussed with particular reference to the variability of the live weights. It is concluded that the weights of cockerels or pullets taken separately should not show a standard deviation very much greater than 15 per cent. of the mean weight for the group, unless the experimental treatment or other conditions have

seriously interfered with the rate of growth. It is pointed out that comparisons of mean weights and of standard deviations may be misleading unless cockerels and pullets are considered separately. Smallest significant differences between the mean weights of groups are tabulated for standard deviations of 15 per cent., 17·5 per cent. and 20 per cent., and for groups of 10 to 100 birds.

REFERENCE.

(1) TITUS, H. W. and JULL, M. A. The growth of Rhode Island Reds and the effect of feeding skim milk on the constants of their growth curves. *J. Agric. Res.* (1928), **36,** 515.

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A NOTE ON WOOD AND CAPSTICK'S METHOD OF CALCULATING THE MAINTENANCE REQUIREMENTS OF THE ADULT SHEEP.

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(With Two Text-figures.)

WHILE engaged in studies relating to the maintenance requirements of chickens at the U.S. Animal Husbandry Experiment Farm, Beltsville, Maryland, the writers became interested in the work reported by Wood and Capstick[4] on the maintenance requirement of the adult sheep. A careful study was made of their method with a view to applying it in the estimation of the maintenance requirements of chickens of various live weights. The results of this study appear to be of sufficient general interest to justify their presentation.

The formula employed by the above-mentioned investigators to estimate the maintenance requirements of adult sheep was written

$$R = Am + Gc, \qquad \text{......(1)}$$

in which

R = daily ration expressed in pounds of starch equivalent,

A = surface area of the animal in square metres calculated by means of the formula:

$$\text{Surface area} = \frac{9 \times (\text{live weight in gm.})^{\frac{2}{3}}}{10,000},$$

m = maintenance requirement per square metre of body surface,

G = daily gain in live weight in pounds,

c = number of pounds of starch equivalent necessary to produce one pound of gain in live weight.

Since the values of A were calculated from the live weights by assuming a direct proportionality between the surface area and the two-thirds power of the live weight of the animal, equation (1) may be written

$$R = aW^{\frac{2}{3}} + cG, \qquad \text{......(2)}$$

in which W is the live weight of the animal and $a = \dfrac{mA}{W^{\frac{2}{3}}}$.

It appeared to the writers that the fit of equation (2) to the data could be improved by substituting a general constant, n, for the exponent $2/3$ and determining its most probable value together with the other two constants in the equation by the method of least squares.

The equation

$$R = aW^n + cG \qquad \qquad \ldots\ldots(3)$$

cannot be fitted by the method of least squares directly, for it is not in a linear form, with respect to its constants. However, it can easily be transformed into a linear form if an approximate value of n is known.

Let n_0 be a known approximate value of n and let α be the correction which must be added to this approximate value to give the true value. Then $W^n = W^{(n_0+\alpha)}$. Expanding by means of Taylor's theorem, the expression $W^{(n_0+\alpha)}$ may be written $W^{n_0} + \alpha \cdot W^{n_0} \cdot \log_e W$, provided α is sufficiently small so that the other terms of the series may be disregarded. Substituting this expression for W^n in equation (3), the equation becomes

$$R = aW^{n_0} + \beta \cdot W^{n_0} \cdot \log_e W + cG, \qquad \ldots\ldots(4)$$

in which $\beta = a \cdot \alpha$.

In this form the equation is linear with respect to the three constants a, β and c, which can be readily determined by the method of least squares. a and c are determined directly and n is calculated by adding the correction, β/a, or α, to the approximate value, n_0.

The writers proceeded to fit equation (4) to Wood and Capstick's data, assuming $2/3$ to be an approximate value of n[1]. It was at once apparent that $2/3$ was much too poor an approximation to the most probable value of n to permit the fitting of equation (4). In order to get a closer approximation to the true value of n, the writers employed a method used by Van Orstrand and Dewey[2].

The method consists simply of fitting equation (3) by arbitrarily assigning some definite value to the constant n, determining the constants a and c by the method of least squares, and calculating the sum of the squares of the differences between the values of R actually observed and the corresponding values calculated from the fitted equation. This procedure is repeated, using different values of n over a range sufficiently great to include the correct value. When the sums of the squares of the residuals are plotted on co-ordinate paper, against the

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[1] The live weights of the animals were not given in Wood and Capstick's paper. The writers recalculated them from the surface areas by means of the formula given for the calculation of surface areas from live weights. They were expressed in pounds to be consistent with the units employed to measure gains in live weight and starch equivalent.

appropriate values of *n*, as abscissae, a U-shaped curve is obtained. The sum of the squares of the residuals will obviously be least for the "best" value of *n*, so one has only to read the abscissa of the lowest point on the curve to learn this "best" value of *n*.

Fig. 1. The effect of using different values of *n* in fitting the equation, $R = aW^n + cG$, upon the sum of the squares of the differences between the calculated rations and the actual rations. Group 1 (sheep which gained weight).

This method was applied to the two sets of data given by Wood and Capstick with the results illustrated in Fig. 1 (sheep which gained weight) and Fig. 2 (sheep which lost weight). From these two graphs the "best" values of *n* may be seen to be approximately − 0·15 and − 0·55, respectively, for the sheep which gained and lost weight.

These approximate values of *n* were repeatedly adjusted by means

of equation (4) until the correction α became negligible. The best fitting equations for the two sets of data are as follows:

Sheep which gained weight:
$$R = 3\cdot087 \ W^{-0\cdot141843} + 0\cdot4988 \ G. \qquad \ldots\ldots(5)$$

Sheep which lost weight:
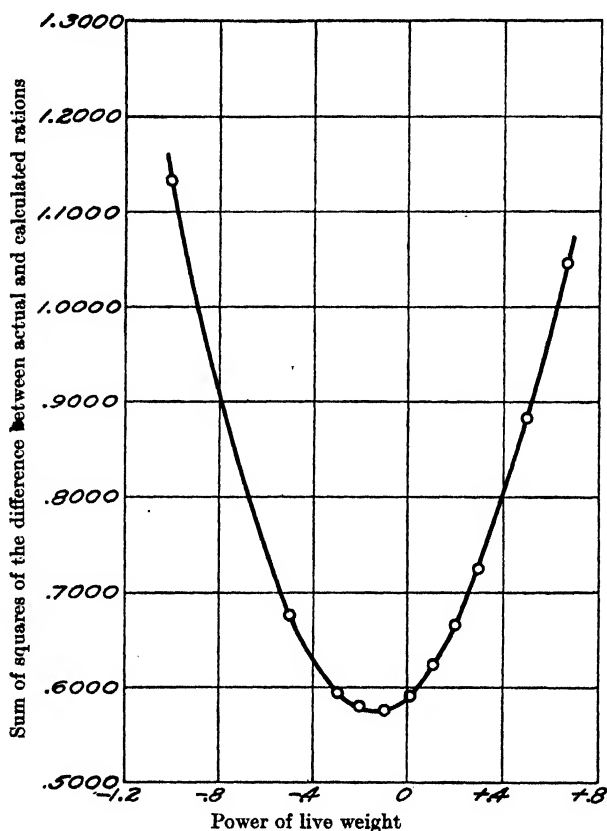$$R = 24\cdot39 \ W^{-0\cdot554083} + 0\cdot9118 \ G. \qquad \ldots\ldots(6)$$
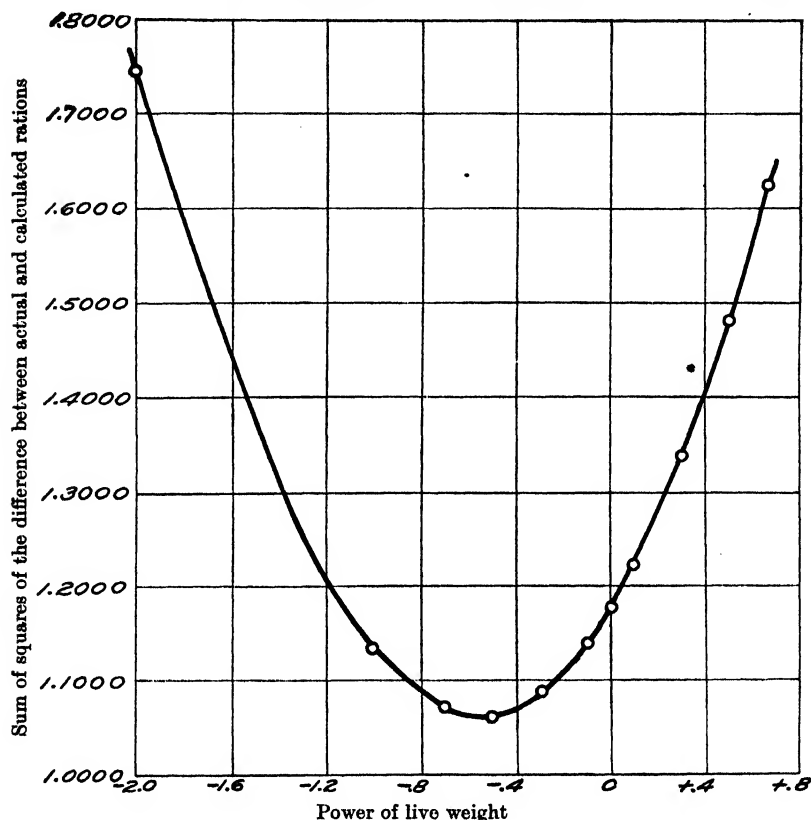
Fig. 2. The effect of using different values of n in fitting the equation, $R = aW^n + cG$, upon the sum of the squares of the differences between the calculated rations and the actual rations. Group 2 (sheep which lost weight).

These results indicated that, for the animals within each group, the differences in maintenance requirements were caused by other factors than differences in surface area. As Wood and Capstick themselves pointed out, the basal metabolism of an animal is but one of the factors contributing to the maintenance requirement. It is also true that, even

if basal metabolism makes up the greater part of the maintenance requirement, the effects of differences in surface area among the experimental animals may be obscured by the variability of the animals with respect to basal metabolism if the differences in surface area are not sufficiently great.

A study of Wood and Capstick's data revealed that the average live weight and surface area of the animals were greater in the group which lost weight than in the group which gained weight. It occurred to the writers that, if the data for the animals in the two groups could be combined, the differences in surface area would be sufficiently great to permit the demonstration of a proportionality between surface area and maintenance requirement.

It was not permissible to fit equation (3) to the combined sets of data because of the change in value of the constant, c, with the decrease in level of nutrition. The ideal method of attack would obviously have been to substitute a suitable variable for the constant c in the equation and thus compensate for the change in utilisation of the feed. The lack of information regarding the relationship between utilisation of feed and level of nutrition made this impossible.

It was decided to obtain an average value of the maintenance requirement per animal for each group separately. Figs. 1 and 2 show that, for a considerable range in value of exponent n, a fit as good, or better, was obtained than when n was arbitrarily fixed at 2/3. When n was taken to be equal to zero, the fit of equation (3) to each set of data was better than when n was taken to be equal to 2/3. Tables I and II show that the value zero gave a fit which was not greatly inferior to that which was obtained when the constants given in equations (5) and (6) were used. It was concluded that, from the standpoint of general physical significance, zero was probably a more rational value of n than those given in the equations which fitted the data best.

It is obvious that when n is equal to zero the value of the expression W^n is unity, regardless of the value of W. In other words, assuming n to be zero is equivalent to assuming a constant value of the maintenance requirement, no matter what the live weight of the animal may be. The equations obtained by assuming n to be equal to zero are:

Sheep which gained weight:
$$R = 1\cdot54484 + 0\cdot517840\,G. \qquad \qquad ......(7)$$

Sheep which lost weight:
$$R = 1\cdot61275 + 1\cdot15615\,G. \qquad \qquad ......(8)$$

The maintenance requirement per animal as given by these equations is 1·54484 pounds of starch equivalent for the animals which gained weight and 1·61275 pounds for those which lost weight. The average surface areas of the animals, obtained by averaging the values given by Wood and Capstick, are 1·360 square metres for the animals which gained weight and 1·447 square metres for those which lost weight. Dividing the average maintenance requirement for each group by the corresponding average surface area gives values of 1·136 and 1·115 pounds of starch equivalent as the maintenance requirement per square metre for the groups which gained and lost weight respectively.

Table I. *The effect of different values of n in the equation, $R = aW^n + cG$, on the values of the calculated rations.*

Group 1 (sheep which gained weight).

Calculated rations and their deviations from actual rations when different values of n were used in the equation

		$n = 0.666667$		$n = -0.141843$		$n = 0.000000$	
	Actual rations	Calculated rations		Calculated rations		Calculated rations	
Sheep No.	Pounds starch equiv.	Pounds starch equiv.	Deviation	Pounds starch equiv.	Deviation	Pounds starch equiv.	Deviation
1	1·94	1·91	+0·03	1·86	+0·08	1·87	+0·07
2	1·94	1·96	−0·02	1·85	+0·09	1·87	+0·07
3	2·01	1·76	+0·25	1·84	+0·17	1·82	+0·19
4	1·58	1·68	−0·10	1·84	−0·26	1·80	−0·22
5	1·58	1·70	−0·12	1·79	−0·21	1·77	−0·19
6	2·01	1·63	+0·38	1·70	+0·31	1·70	+0·31
7	1·58	1·87	−0·29	1·65	−0·07	1·70	−0·12
8	1·15	1·81	−0·66	1·63	−0·48	1·67	−0·52
9	1·83	1·70	+0·13	1·66	+0·17	1·67	+0·16
10	1·83	1·60	+0·23	1·61	+0·22	1·62	+0·21
11	1·66	1·62	+0·04	1·61	+0·05	1·62	+0·04
12	1·57	1·33	+0·24	1·66	−0·09	1·61	−0·04
13	1·58	1·78	−0·20	1·55	+0·03	1·60	−0·02
14	1·61	1·27	+0·34	1·64	−0·03	1·58	+0·03
Sum of squares of deviations		1·0369		0·5762		0·5855	
Root-mean-square deviation		±0·2722		±0·2029		±0·2045	

These values do not differ greatly from those found by Wood and Capstick, viz. 1·07 and 1·13 pounds of starch equivalent, respectively. However, the difference between the writers' values for the two groups is only 0·021 pound, or 1·9 per cent., as contrasted with a difference of 0·06 pound, or 5·5 per cent., between Wood and Capstick's values. It is also interesting to note that in the case of the writers' values the

maintenance requirement is greater for the animals which gained weight whereas the opposite is true of Wood and Capstick's values.

Wood and Capstick held the view that the maintenance requirement per square metre of body surface should be the same regardless of whether the animals gained or lost weight. The writers suggest that, since the heat production of an animal is increased by raising its level of nutrition, the maintenance requirement of an animal which is receiving sufficient feed to gain weight should be somewhat higher than that of an animal which is losing weight.

Table II. *The effect of different values of n in the equation, $R = aW^n + cG$, on the values of the calculated rations.*

Group 2 (*sheep which lost weight*).

Calculated rations and their deviations from actual rations when different values of n were used in the equation

Sheep No.	Actual rations Pounds starch equiv.	$n = 0\cdot666667$ Calculated rations Pounds starch equiv.	Deviation	$n = -0\cdot554033$ Calculated rations Pounds starch equiv.	Deviation	$n = 0\cdot000000$ Calculated rations Pounds starch equiv.	Deviation
15	1·57	1·42	+0·15	1·77	−0·20	1·61	−0·04
16	1·50	1·62	−0·12	1·58	−0·08	1·61	−0·11
17	1·15	1·78	−0·63	1·46	−0·31	1·61	−0·46
18	1·66	1·55	+0·11	1·54	+0·12	1·55	+0·11
19	1·94	1·49	+0·45	1·56	+0·38	1·54	+0·40
20	1·61	1·34	+0·27	1·70	−0·09	1·54	+0·07
21	1·34	1·50	−0·16	1·54	−0·20	1·53	−0·19
22	1·50	1·49	+0·01	1·50	0·00	1·51	−0·01
23	1·47	1·49	−0·02	1·32	+0·15	1·40	+0·07
24	1·34	1·35	−0·01	1·44	−0·10	1·40	−0·06
25	1·94	1·20	+0·74	1·33	+0·61	1·28	+0·66
26	1·47	1·43	+0·04	1·16	+0·31	1·28	+0·19
27	0·68	1·26	−0·58	1·11	−0·43	1·17	−0·49
28	0·73	0·73	−0·05	0·86	−0·18	0·79	−0·11
Sum of squares of deviations		1·6356		1·0674		1·1709	
Root-mean-square deviation		±0·3417		±0·2761		±0·2892	

The writers' values for the maintenance requirement, per square metre of body surface, for the two groups of sheep are in agreement with their suggestion, but the evidence is by no means conclusive. The average maintenance requirements, per animal, as given in equations (7) and (8), are subject to a certain degree of unreliability. Also, the formula for the calculation of surface area from live weight, given by Wood and Capstick, may not describe the relation between surface area and live weight accurately in the case of their experimental animals.

The work of Brody and associates[1] has shown that surface area is not always proportional to the two-thirds power of live weight. In some cases the value of the exponent may be much lower. Brody has found values as low as 0·32 in the case of animals of nearly the same height but of different live weights.

The writers desired to learn what value of the exponent would have to be substituted for 2/3 in Wood and Capstick's surface area formula in order to make the calculated maintenance requirement per square metre of body surface the same for the two groups of sheep. This was accomplished as follows.

Let kW^n represent the surface area of one animal. Then the sum of the surface areas of the fourteen animals in one group is $k.\Sigma W^n$ and the average surface area is $\dfrac{k.\Sigma W^n}{14}$. Designating the average surface area of the animals which gained weight by $\dfrac{k.(\Sigma W^n)_1}{14}$ and the average surface area of those which lost weight by $\dfrac{k.(\Sigma W^n)_2}{14}$, the maintenance requirements per square metre for the two groups may be written

$$\frac{1\cdot54484 \times 14}{k.(\Sigma W^n)_1} \text{ and } \frac{1\cdot61275 \times 14}{k.(\Sigma W^n)_2},$$

respectively. If the maintenance requirements, per square metre of body surface, of the two groups of animals are equal,

$$\frac{1\cdot54484 \times 14}{k.(\Sigma W^n)_1} = \frac{1\cdot61275 \times 14}{k.(\Sigma W^n)_2} \text{ or } \frac{(\Sigma W^n)_2}{(\Sigma W^n)_1} = \frac{1\cdot61275}{1\cdot54484} = 1\cdot04396.$$

A series of values of the ratio $\dfrac{(\Sigma W^n)_2}{(\Sigma W^n)_1}$ was calculated by using different values of n ranging from 0·0 to 1·0. These ratios were plotted on co-ordinate paper, against the values of n by means of which they were calculated, as abscissae. The resulting curve was almost a perfectly straight line. The value of n which gave the ratio a value of 1·04396 was found by interpolation to be 0·4568.

The interpretation of this result resolves itself into an evaluation of the validity of the exponent 2/3 in Wood and Capstick's surface area formula. Their assumption that the maintenance requirement should be the same for both groups of sheep, per square metre of body surface, appears to be valid if one is inclined to favour the view that surface area is, in this case, proportional to live weight raised to a lower power than 2/3. In the light of Brody's work, it is possible that, in the case of

the sheep used in Wood and Capstick's study, the value of the exponent in the surface area formula should have been about 0·4568 instead of 2/3. At this time it is impossible to say whether or not this is actually the case. More information is needed regarding the true relationship between surface area and live weight in the case of sheep.

The writers are inclined to suspect that 2/3 is too large a value for the exponent in Wood and Capstick's surface area formula, but are not prepared to offer the value 0·4568 as a better approximation to the true value. The writers are merely pointing out that a value as low as this one is well within the range in value of the exponent found by Brody and his co-workers, but they see no necessity for arbitrarily postulating the same maintenance requirement, per square metre of body surface, for both groups of sheep. On the other hand, it seems more logical to them to consider the maintenance requirement of an animal, as the term is used here and in Wood and Capstick's paper, as being a function of live weight, "condition," plane of nutrition, and activity of the animal, rather than of the surface area and activity of the animal as suggested by Wood and Capstick.

It appeared desirable to have some idea of the reliability of the values of the constants obtained when equation (3) was fitted to each set of data, with n equal to the "best" value, to 0·0, and to 2/3, respectively. Approximate values of the probable errors of the constants were calculated in the following manner. A value of the constant, of which the probable error was desired, was calculated for each set of observed values of R, W and G by substituting these values of R, W and G in equation (3), together with the values of the two other constants. A value of the constant was thus calculated for every set of observed values of R, W and G. These calculated values of the constant were assumed to be such that their arithmetic mean was equal to the value of the constant obtained when equation (3) was fitted by the method of least squares. The probable error of the constant was calculated by the well-known method of calculating the probable error of an arithmetic mean. The probable errors of the constants, calculated by this method, are given in Tables III and IV.

The most noticeable feature of the probable errors of the constants is the high probable error of c in each case. Wood and Capstick pointed out that the values of this constant were subject to a high degree of unreliability but made no attempt to determine the extent of the error statistically. The probable errors of c are, in fact, so large as to lead one to question the significance of the difference between the values of this constant obtained from the two sets of data.

Table III. *Probable errors of the constants in the equation, $R = aW^n + cG$, when different values of n were used in fitting the equation.*

Group 1 (sheep which gained weight).

Constant	Value of constant	Probable error of constant	Ratio of constant to probable error
Equation giving best fit to data:			
a	+3·087	±0·0761	40·57
n	−0·141843	±0·0054183	26·18·
c	+0·4988	±0·15701	3·18
Equation obtained with $n = 0$:			
a	+1·54484	±0·038476	40·15
n	—	—	—
c	+0·517840	±0·1569938	3·30
Equation obtained with $n = 2/3$:			
a	+0·0565501	±0·00196108	28·84
n	+0·666667	±0·0075574	88·21
c	+0·776129	±0·3672743	2·11

Table IV. *Probable errors of the constants in the equation, $R = aW^n + cG$, when different values of n were used in fitting the equation.*

Group 2 (sheep which lost weight).

Constant	Value of constant	Probable error of constant	Ratio of constant to probable error
Equation giving best fit to data:			
a	+24·39	±0·817	29·74
n	− 0·554033	±0·0066244	83·64
c	+ 0·9118	±0·513013	1·78
Equation obtained with $n = 0$:			
a	+ 1·61275	±0·054280	29·71
n	—	—	—
c	+ 1·15615	±0·524819	2·20
Equation obtained with $n = 2/3$:			
a	+ 0·0601952	±0·00230881	26·07
n	+ 0·666667	±0·0078203	85·25
c	+ 1·39509	±0·625155	2·23

The writers do not agree with Wood and Capstick's suggestion that the value of the constant c depends upon whether the animals gained or lost weight. If there were an increased economy of utilisation of the body tissues when an animal is losing weight, the writers would expect to find the effects of this economy manifested in a lower maintenance requirement, rather than in a higher value of c.

It appears to the writers that, in order to obtain a value of c which is not excessively variable, the experimental animals must be in approximately the same condition, *i.e.* of the same degree of fatness. The value of c would seem to depend upon the chemical composition of the gains or losses in live weight, rather than on whether tissue is being added to,

or lost from, the body of the animal. The value of c should be greatest when the tissues which are added or lost are of such chemical composition as to have the highest calorific value per unit weight of material, *i.e.* when they contain the highest percentage of fat.

This view of the equivalence of feed and body tissues agrees with the recent findings of Wiegner and Ghoneim(3) in their work on the net energy of feeding stuffs in relation to the amount of metabolisable energy in the daily ration. However, it is conceivable that an animal could be in such a state of nutrition that an increase in the level of feed intake would cause the animal to build tissues containing a percentage of fat greater than that which is present in those tissues which would be lost if the level of feed intake were decreased sufficiently to cause the animal to lose weight. For an animal in such condition a unit gain in weight would be equivalent to a larger amount of feed than a unit loss in weight.

The writers believe that it would have been more logical for Wood and Capstick to have classified their animals according to condition, rather than according to whether they gained or lost weight. As a matter of fact, the animals appear to have accidentally fallen into such a classification to some extent, for the average live weight of the sheep in the group which lost weight was higher than that of the sheep in the group which gained weight. It is possible that this higher average live weight was, in part, due to some of the animals being better fleshed and containing higher percentages of fat in their bodies. If this was actually the case, as seems highly probable, the higher value of the constant c for the group of sheep which lost weight is what might be expected.

The slight negative correlation between live weight and maintenance requirement, indicated by equations (5) and (6), suggests that the animals within each group were rather variable with respect to condition. The higher live weights of some of the animals in each group may have been due to the animals being in better condition, and do not necessarily imply greater surface areas. If the animals in each group varied considerably with respect to condition, one would expect a high probable error of the constant c, as was found to be the case.

SUMMARY AND CONCLUSIONS.

The writers do not mean to infer that the maintenance requirement of the adult sheep, as calculated by Wood and Capstick, is incorrect. The value obtained by these investigators is probably as reliable as any other which could be calculated from their data. The writers fully agree

that Wood and Capstick's value is probably more accurate than previously estimated values of the maintenance requirement of the adult sheep.

However, the writers' analysis of Wood and Capstick's data shows that, when the range in live weight of the experimental animals is small, there is little justification for attempting to refine the calculations by assuming a proportionality between the maintenance requirement and the two-thirds power of the live weight of the animal. This does not mean that the maintenance requirement of an animal is not affected by its surface area, but that the effects of small differences in surface area may be obscured by the effects of other factors contributing to the maintenance requirement.

The writers believe that the maintenance requirement of an animal is influenced, to some extent, by the level of feed intake as well as by the size and activity of the animal. When the range in live weight of the experimental animals is small, the effects of all the factors contributing to the maintenance requirement may be such that the higher maintenance requirements tend to be associated with the lower live weights, as was apparently the case in each group of Wood and Capstick's animals.

There is also some possibility that the lack of a proportionality between the maintenance requirement and the two-thirds power of the live weight is due to the fact that the surface area is, in some cases, not proportional to the two-thirds power of the live weight. If the animals are not uniform with respect to condition, a heavy animal will not necessarily have a correspondingly greater surface area than a somewhat lighter animal.

The writers believe that the chemical composition of gains or losses in live weight chiefly determines the amount of feed to which they are equivalent, and the chemical composition of these changes in live weight is dependent, at least in part, upon the condition of the animal.

In the case of Wood and Capstick's analysis of their data, it might have been more logical for them to have classified their animals on the basis of condition, rather than on the basis of gain or loss in live weight, provided, of course, that they had sufficient information regarding the condition of the animals at hand to make such a classification possible.

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STUDIES IN TROPICAL SOILS.

II. SOME CHARACTERISTIC IGNEOUS ROCK SOIL PROFILES IN BRITISH GUIANA, SOUTH AMERICA.

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INTRODUCTORY.

IN an attempt to trace the transformations of the sesquioxide and silica components of igneous rocks subjected to prolonged action by agents of alteration and weathering under humid tropical climatic conditions, a detailed study has been made of some characteristic soil profiles in British Guiana. The method of study comprised both quantitative chemical analysis, rapid soil examination, and the application of an alizarin-adsorption procedure [4] previously elaborated by one of us (F. H.). In addition, comparisons have been made with the results of some very detailed petrographical and chemical determinations performed some years ago by the late Sir John Harrison on material collected in the same localities. Harrison's results are described in part in papers already published [6, 7, 8], but the greater portion of his data is contained in an unfinished and unpublished manuscript entitled "Field and laboratory notes on the katamorphism of igneous rocks under tropical and temperate conditions" (pp. 1–243, Tables I–CI), apparently written between 1920 and 1925, and originally intended as part of a new issue of the *Geology of the Goldfields of British Guiana* [5][1].

PART I. PETROGRAPHICAL AND CHEMICAL ASPECTS.

1. THE GENERAL GEOLOGY OF BRITISH GUIANA.

The geology of British Guiana is relatively simple. The western and central regions are occupied by a *Sedimentary Series* (the Kaieturian sandstone), of Palaeozoic or Cretaceous age, forming an elevated plateau

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[1] The writers desire to express indebtedness to His Excellency Sir Edward Denham, the Governor of British Guiana, and to Prof. J. S. Dash, Director of Agriculture, for permission to abstract and publish those portions of the late Sir John Harrison's manuscript that have bearing on their own investigations.

ending eastwards in a conspicuous escarpment. The sedimentary rocks contain later intrusions of basic igneous rocks. The rest of the area (with the exception of the coastal alluvial clay-belt comprising the sugar-estates) is an undulating dissected peneplane composed of an Archaean *Gneiss Granite Basement Complex*, associated with a *Volcanic Series*. The gneiss resembles the Laurentian; the clastic members of the volcanic series resemble the "greywacke" rocks of north-east Canada. Intruded into the gneiss-granite-volcanic series also occur more recent basic igneous rocks which have been metamorphosed into epidiorites and hornblende schists. Still later intrusions are granitic in type, and include porphyrites, porphyries, aplites, syenites and diorites. These intrusions are of greater age, however, than the sedimentary series which unconformably overlies the Archaean basement. The peneplane is everywhere covered by forest-clad residual deposits comprising low hills of white sand, vast masses of siliceous clays and kaolins, thick layers of red earths, and surficial accumulations of laterite, ironstone and bauxite.

The total area of British Guiana is some 89,500 square miles, *i.e.* about the size of Great Britain, but the main rock types and geological formations occurring within the region are represented over a very much larger area comprising part of Venezuela, Dutch Guiana, French Guiana, and part of Brazil.

2. Description of the Profiles.

Five profiles, representative of sedentary soils overlying the main igneous rock types of British Guiana, were examined. The last (E) is representative of the Archaean basement gneiss complex, and the rest, of the various later intrusions.

Name of profile	Parent rock	Total silica content (%)	Class
(A) Tiger Hill—red clay	Hornblende schist	50	Basic
(B) Hope Quarry	Dolerite	51	Basic
(C) Blue Mountain Quarry	Quartz diorite	60	Intermediate
(D) Mazaruni Quarry	Granite	73	Acidic
(E) Kamwatta Creek—sandhill	Gneissose granite	73	Acidic

The partial bulk compositions of the parent rocks, expressed on the *quartz-free* basis, are:

	SiO_2 (combined) %	Al_2O_3 %	Fe_2O_3 %	CaO %	MgO %
(A) Hornblende schist	47·5	12·8	10·4	12·3	10·0
(B) Dolerite	51·3	15·8	10·3	9·1	7·7
(C) Quartz diorite	32·3	27·4	13·0	10·7	tr.
(D) Granite	60·2	21·2	2·8	1·5	1·2
(E) Gneissose granite	55·8	26·4	4·0	5·3	1·1

(A) *Tiger Hill profile—red clay.*

This profile was exposed in a pit excavated in the highland extending from Tiger Hill to Arukami Hill in the North-West District of British Guiana, at elevation 600 feet above sea-level(2).

The chief minerals present in the *parent rock* (hornblende schist) are: blue hornblende (55 per cent.), plagioclase felspar (labradorite—39 per cent.), quartz (5 per cent.), and titaniferous magnetite (0·5 per cent.). The rock surface is broken into fragments of all sizes. The soil profile, described from below upwards, consists of the following divisions:

1. *"Primary laterite,"* thickness 3 inches, hard and crusty.

2. *Tawny red earth*, thickness 10 to 20 feet, friable and very gravelly, especially in its upper part. The gravel consists of pieces of laterite, pellets of ironstone and particles of quartz.

3. *Topsoil*, thickness 9 inches; red-brown humic clay.

4. *Surficial laterite and ironstone*, frequently vesicular and slaggy, scattered in blocks over the soil surface.

Microscopical examinations made by Harrison show that the inner adherent layer ($\frac{1}{4}$ inch) of the *primary laterite* contains a large proportion of flaky *gibbsite* ($Al_2O_3 . 3H_2O$), together with unaltered particles of felspar and hornblende. The second layer ($\frac{3}{4}$ inch) consists mainly of gibbsite (partly in the form of tiny spheroids), hydrated iron oxide, titania, and minute grains of quartz. The third layer (2 inches) contains little gibbsite, but much kaolin. The fourth, outer crusty layer ($\frac{1}{8}$ inch) consists mainly of kaolin and limonite. The *red earth* consists chiefly of kaolin (50 per cent.), together with a significant amount of gibbsite (6 per cent.), quartz (22 per cent.), and iron oxides (21 per cent.). The larger pieces of *included gravel* are composed mainly of aggregates of kaolin, with some limonite, quartz and titania. The finer gravel and ironstone pellets contain sperulitic gibbsite as well as kaolin and iron oxides.

The results of chemical analysis of materials representative of the different layers are presented in Table I. Our own samples comprised only surface soil and red gravelly clay. The rest of the data are abstracted from Harrison's records.

(B) *Hope Quarry profile.*

The site of this profile is an old disused road-metal quarry situated 100 yards from the left bank of the Demerara River, 80 miles south of Georgetown, at an altitude of 10 feet above sea-level. It lies within the forest-clad undulating peneplane.

Table I. *Results of chemical analyses and alizarin tests.*

| | | | Chemical composition | | | | | | | Alizarin values | | | | | | | |
| | | | Combined | | | | | | | Material | | Oxalate extract | | Corrected | | Free Al₂O₃ (%) | Free (active) Fe₂O₃ (%) |
Description of profile	Lower depth (in.)	Quartz (%)	SiO₂ (%)	Al₂O₃ (%)	Fe₂O₃ (%)	TiO₂ (%)	CaO (%)	MgO (%)	H₂O (%)	Fresh	Ign.	Fresh	Ign.	Fresh Factors	Ign.	0·150	0·066 0·040
(A) Tiger Hill profile (hornblende schist):																	
DRA 27. Red-brown humic clay (3)	9	40·6	13·5	11·6	18·5	4·2	Nil	Nil	7·9	100	54	18	28	82	26	3·9	5·4
28. Tawny red gravelly clay (2)	27	31·0	17·0	15·9	20·9	3·8	,,	,,	8·8	111	42	12	18	99	24	3·6	0·5
29, 30. Tawny red gravelly clay (2)	63	24·0	21·1	18·4	20·5	3·6	,,	,,	9·6	112	29	16	10	96	19	2·8	6·4
HS 9. Primary laterite (1)	—	5·0	7·4	34·8	24·9	4·9	0·1	1·6	20·8	—	—	—	—	—	—	—	—
10. Parent rock	—	4·3	45·5	12·2	10·0	4·6	11·8	9·5	0·4	—	—	—	—	—	—	—	—
(B) Hope Quarry profile (dolerite):																	
DRA 19. Red-brown topsoil (4)	21	39·0	16·9	15·5	14·5	2·2	0·1	0·1	12·6	86	46	25	30	61	16	2·4	2·4
20. Red earth (3)	43	11·5	29·2	24·0	20·8	1·4	0·5	0·3	13·4	113	43	12	9	101	34	5·1	4·0
21, 22. Red mottled earth (2)	106	5·6	29·5	26·0	22·8	1·5	0·7	0·3	14·2	117	54	11	10	105	44	6·6	7·0
23. Tawny yellow earth (2)	110	11·8	23·0	22·9	17·6	0·7	8·4	6·5	8·2	61	29	11	14	50	15	2·3	3·3
24. Pale yellow earth (2)	112	14·6	23·4	19·3	17·8	1·0	6·5	6·9	7·2	46	21	8	10	38	11	1·7	2·5
25. Primary laterite (1)	—	9·3	27·7	24·6	19·0	1·0	3·9	3·8	10·3	73	27	8	10	65	17	2·6	4·3
26. Parent rock	—	0·1	51·1	15·7	10·3	1·7	9·1	7·7	0·4	23	12	14	9	9	3	0·5	0·6
(C) Blue Mountain Quarry (quartz diorite):																	
DRA 1. Dark grey topsoil (7)	17	74·6	8·7	1·7	6·7	1·3	1·0	—	5·6	35	25	30	10	5	15	2·3	0·2
2. Pale brown subsoil (8)	29	61·5	13·9	10·6	5·6	1·3	0·8	—	6·5	68	29	12	8	56	21	3·1	2·2
3–7. Orange and red earth (5, 4)	62½	55·7	16·0	12·3	7·6	1·0	0·4	—	7·5	94	36	10	9	84	26	3·9	5·2
9. Primary laterite (3)	63	47·2	11·7	18·8	8·3	0·8	2·8	—	9·7	120	63	13	14	107	49	7·4	7·1
11–15. Rotten rock (2, 1)	71¾	51·0	12·3	9·8	12·8	0·8	4·7	—	7·9	78	42	14	11	64	31	4·7	2·5
17. Parent rock	—	40·2	19·3	16·4	7·8	1·3	6·4	—	0·6	16	12	6	6	10	6	0·9	0·4
(D) Mazaruni Quarry profile (granite)—mainly after Harrison:																	
HS 1. Topsoil (5)	8	38·9	29·8	23·2	1·6	—	0·0	0·0	4·7	—	—	—	—	—	—	—	—
2. Pale grey subsoil (4)	14	29·7	31·4	25·3	1·2	—	0·0	0·0	10·3	—	—	—	—	—	—	—	—
3. Granite subsoil (3)	30	32·1	30·8	24·1	1·7	—	0·0	0·3	8·0	—	—	—	—	—	—	—	—
4. Granite sand (3)	146	37·3	29·8	21·2	2·0	—	0·1	0·3	6·4	—	—	—	—	—	—	—	—
5. Granite sand (3)	152	41·7	28·5	18·8	2·0	—	0·1	0·2	5·2	—	—	—	—	—	—	—	—
6. Softened shaley rock (2)	175	41·2	32·7	15·1	2·3	—	0·2	0·4	1·7	—	—	—	—	—	—	—	—
7. Parent rock	—	31·7	41·1	14·5	1·9	—	1·0	0·8	0·7	—	—	—	—	—	—	—	—
DRA 56. Granite sand (3)	—	42·1	20·6	22·6	2·9	—	0·1	0·7	6·4	29	8	19	8	10	0	0·0	0·7
(E) Kamwatta Creek (gneissose granite):																	
DRA 31. Surface mat, sepia sand (2)	2	81·9	7·0	4·1	1·0	0·2	Nil	Nil	2·6	52	14	33	7	19	7	1·1	0·8
32. Grey sand (2)	20	75·5	9·5	7·3	3·1	0·2	,,	,,	3·5	58	10	28	6	30	4	0·6	1·2
33. Pale brown sandy clay	23	60·2	15·7	13·4	3·5	0·2	,,	,,	5·3	77	16	24	13	53	3	0·8	2·1
34. Brownish buff clay	28	43·2	23·7	20·6	3·1	0·3	,,	,,	7·8	92	14	20	10	72	4	0·8	2·9
35. Orange-pink clay (1)	76	37·3	26·4	23·4	3·3	0·2	,,	,,	8·6	91	13	14	10	77	3	0·5	3·1
HS 8. Parent rock	—	37·8	34·7	16·2	2·3	0·2	3·3	0·7	0·1	—	—	—	—	—	—	—	—

The *parent rock* (quartz-free dolerite) consists mainly of plagioclase felspar (labradorite—50 per cent.), colourless augite (42 per cent.), enstatite (2 per cent.), titaniferous magnetite (4·5 per cent.), greenish biotite (1 per cent.) and olivine (0·4 per cent.).

The profile, described from below upwards, shows the following sequence:

1. *"Primary laterite,"* thickness 2½ inches, lying directly over the parent rock, or, where detached from it, embedded in pale yellow earth.

2. *Ochreous yellow clay*, mottled red in places, thickness 18 to 22 inches, incoherent and friable.

3. *Red earth*, thickness 60 to 120 inches, incoherent and friable.

4. *Topsoil*, 21 inches deep, loose and sandy; colour, red-brown, becoming paler and greyer in the surface humic layer.

5. *Surficial ironstone*, occasionally occurring in blocks above or within the sandy topsoil.

Petrographic examinations made by Harrison show that near the surface of the parent rock the cleavage planes of the minerals are eroded and contain scales of gibbsite. At the junction of the rock with its *primary laterite*, felspar occurs as fragments surrounded by gibbsite; augite as chlorite aggregates or reticulations of oxide of iron resembling goethite. (No secondary mica could be detected.) The *ochreous clay*, overlying the hard laterite crust, is composed mainly of amorphous kaolin, limonite and secondary crystalline quartz. Gibbsite is stated to be scarce or absent. The red *earths* resemble the ochreous clay, but they contain less kaolin and more limonite. The sandy *topsoil* consists mainly of quartz (80 per cent.), together with kaolin (10 per cent.) and hydrated iron oxides (10 per cent.). The *surficial ironstone* is composed of iron oxides (65 per cent.), gibbsite (26 per cent.) and kaolin (5 per cent.). The results of our chemical analyses of these materials are also contained in Table I.

(C) *Blue Mountain Quarry profile.*

The site of this is another disused quarry situated ¼ mile east of the last, further down stream, and at the same elevation.

The *parent rock* (epidote quartz diorite) consists of glassy quartz (40 per cent.), plagioclase felspar (andesine—28 per cent.), blue-green hornblende (18 per cent.), brown epidote (9 per cent.), greenish biotite (4 per cent.) and titaniferous magnetite (1 per cent.).

The soil profile presents the following divisions:

1. *Flaky crust*, ⅓ inch thick, overlying the parent rock.

2. *Rotten rock*, thickness 8 to 23 inches, pale creamy yellow in colour, and exhibiting alternating hard and soft scaly layers, 50 to 150 in number.

3. *Quartziferous "primary laterite,"* $\frac{1}{2}$ inch thick, hard and crusty.

4. *Ochreous earth*, thickness 3 inches, tawny yellow in colour, friable, and containing rounded fragments of residual quartz and detached pieces of laterite.

5. *Orange and pale red sandy clay*, thickness 31 inches.

6. *Subsoil*, thickness 12 inches, pale tawny brown in colour, coherent, and sandy in texture.

7. *Topsoil*, thickness 17 inches, loose and sandy, dark grey. at the surface, becoming paler and more brown in colour when traced downwards.

Harrison's petrographical determinations show that the felspar crystals in the *rotten rock* contain gibbsite as cleavage linings, and that complete conversion of felspar into gibbsite has occurred in the layer of primary laterite. Here also hornblende has been changed into limonite. The *ochreous earth* contains, in addition to gibbsite, limonite, and quartz, much ferruginous kaolin and halloysite. In the overlying *orange and red sandy clay*, and in the *subsoil*, gibbsite is less conspicuous, and the material appears to consist mainly of limonite, kaolin and quartz. The *topsoil* is composed chiefly of quartz grains, together with limonite, kaolin and halloysite cemented by limonite.

Data obtained by chemical analysis of samples of the different layers are contained in Table I. In order to save space, certain of the detailed results are omitted; means of values instead are recorded.

(D) *Mazaruni Quarry profile*.

The site is a quarry at the Penal Settlement on the Essequebo River, near the confluence of its tributaries, the Mazaruni and the Cuyuni Rivers, two miles west of Bartica, and about 40 miles from the mouth of the Essequebo River.

The *parent rock* (a typical grey granite) consists of orthoclase felspar (53 per cent.), plagioclase felspar (oligoclase—11 per cent.), quartz (32 per cent.), muscovite mica (3 per cent.) and biotite mica (1 per cent.).

In most sections the granite is seen passing abruptly into a pipe-clay subsoil of no great thickness, overlain by sandy soil. The soil profile thus consists of a grey, leached, humic topsoil, of depth some 6 inches, merging into a whitish grey sandy clay layer of about the same thickness, and then very sharply into hard, more or less iron-stained rotten rock,

which retains the structure of the parent material. Sections exhibiting gradual transition between unaltered granite and incoherent subsoil are seldom seen, and, at the time of our visit, none was visible, but fortunately Harrison, in 1919, was able to examine at the Mazaruni Quarry a deep cutting exposed during blasting operations. The following sequence is summarised from Harrison's detailed notes:

1. The parent rock is covered by a fairly hard and coherent layer, 30 inches in thickness, of *disintegrating granite*, brown in colour at the base, but paler above.

2. The disintegrating rock passes abruptly into a soft mass 6 inches thick, weathered into *shaley layers* of greyish white colour. This material easily crumbles into gravelly sand.

3. A deep layer (some 128 inches thick) of *"granite sand"* next follows. Its lower part still shows the structure of the parent rock, and is more or less coherent and grey in colour. Its upper part is devoid of structure, though still compact, and its colour is streaky pale brownish grey to reddish grey.

4. Above the granite sand lies a pale grey sandy clay *subsoil*, of thickness 6 inches.

5. This is followed by a pale, sandy, humic *topsoil*, of thickness 6 to 8 inches.

Harrison's microscopical examination of thin slices prepared from samples representing the lower part of the zone of *disintegrating granite* showed felspar undergoing fracture and corrosion, with the formation of amorphous, finely divided kaolin. Biotite was completely converted into chlorite and epidote, but muscovite remained unaltered. *Neither gibbsite nor sericitic mica could be detected* among the secondary products of alteration.

Examination of specimens representing the less coherent *shaley layers* showed, in the coarser fractions, the presence of grains of quartz, fragments of orthoclase, plates of plagioclase, shreds of biotite or chlorite, and cloudy plates of muscovite. In addition, the coarser fractions of the lower shaley layers and the upper layer of the underlying disintegrating granite contained spherules of secondary opaline silica, doubtless carried down by percolating water. Here plagioclase and biotite were scarce, orthoclase fragments were reduced in size, and muscovite contained inter-laminar deposits of brown limonite. In the material comprising the *granite sand*, secondary silica was of rare occurrence. Felspars were scarcer in the upper layers, and muscovite, though plentiful, was friable, easily crumbling into powder. Colloidal halloysite and amorphous

kaolin were very abundant throughout the whole mass of granite sand. The *subsoil* consisted mainly of aggregates of kaolin, quartz grains, and a few orthoclase remnants. The *topsoil* consisted mainly of quartz grains, together with kaolin.

The results of chemical analyses, mainly abstracted from Harrison's records, are presented in Table I. Unfortunately we were unable to discover a section so complete in detail as that described by Harrison. The only material collected by us at the Mazaruni Quarry was a sample of *granite sand* immediately overlying hard but partly rotten rock.

(E) *Kamwatta Creek profile—sandhill.*

The profile was exposed in a pit dug in undulating land in the North-West District at elevation 200 feet[2]. The parent rocks comprising this area are Archaean acidic gneisses and gneissose granites, traversed by dykes of dolerite outcropping in flat-topped hills. The chief minerals present in the gneissose granite are: plagioclase felspar (andesine—52 per cent.), granulitic quartz (38 per cent.), hornblende (4 per cent.), ortho-clase (potash) felspar (2 per cent.), secondary muscovite (2 per cent.) and chloritised biotite (2 per cent.). The felspars occur in microperthitic plates containing veins of sericitic mica.

The soil profile is simple, consisting of (1) a bright orange-pink coherent sandy clay (*rotten rock*) overlying the parent gneiss, and merging upwards into (2) a deep, loose, pale buff-coloured quartzose *sandy soil*, tinged dark grey by organic matter. The rotten rock is reached at a depth of about 20 inches.

Analytical data for this profile are contained in Table I.

3. METHODS OF ANALYSIS.

The samples representative of the various layers distinguishable in the soil profiles were examined by conventional methods for the accurate complete chemical analysis of silicate minerals and earthy materials. In every case the *whole* material was analysed; no attempt was made to separate the "clay fraction." Silica was invariably determined in both its forms, namely, combined silica and free quartz, by a modification of a method perfected and employed for many years by the late Sir John Harrison in the Government Laboratory in British Guiana[1]. This procedure was thought to be preferable to an analysis of the separated clay fraction as advocated by Martin and Doyne[9], in view of the facts

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[1] Method for determining combined silica; see Appendix A.

(a) that it is at least as expeditious as clay separation; (b) that the coarser ("sand") fractions of many of the materials examined evidently contain concretionary particles of gibbsite, iron oxides and quartz, whose presence is believed to signify "lateritisation." The deliberate removal and exclusion of these characteristic products might consequently obscure the true implications of results obtained by chemical examination.

In addition to chemical analysis, the materials were examined by the alizarin adsorption method (4).

4. DISCUSSION OF THE PETROGRAPHICAL AND CHEMICAL DATA FOR THE DIFFERENT PROFILES.

In order to compare the profiles from the point of view of kata-morphosis, various ratios have been calculated from the generalised chemical data; they are set out in Table II, in which the profiles are arranged in order of increasing silica content ("acidity") of the parent rock. For each profile, the ratios for the different samples are grouped in six columns, showing the main changes that have occurred between unaltered rock and final surface soil. Harrison's data are used for calculating ratios only in those cases where we were unable to procure material for analysis. (In the table, our samples are denoted DRA; those collected by Harrison are denoted HS.)

It is evident from a comparison of the petrographical and chemical features of the different profiles that each profile may be divided into three main zones which may be designated (i) *Zone of alteration*, (ii) *Zone of secondary changes*, and (iii) *Zone of leaching*. In the narrow zone of alteration (both commencing and completed), the original minerals com-posing the parent rock are more or less profoundly altered into various new products. In the broad zone of secondary changes, further modifica-tions, including some that are synthetic, have affected these products, and finally, in the zone of leaching, partial or complete removal of certain secondary components has occurred, so that the more resistant minerals alone are left in the topsoil.

The following main conclusions are derived from a scrutiny of the chemical data (Table I) and the ratio values (Table II).

(i) *Zone of alteration.*

1. In every case there is a more or less marked decrease in the silica ratios (Nos. i, ii, iii) between the parent rock and the material com-prising the zones of alteration. This implies loss of *combined silica* with concomitant accumulation of sesquioxides and possibly secondary quartz,

Table II. *Comparison of various ratios derived from chemical data for the different profiles.*

	Parent rock	Zone of alteration Com-mencing	Zone of alteration Com-pleted	Zone of secondary changes	Transition	Zone of leaching Topsoil
Basic rocks.						
(A) *Tiger Hill profile (hornblende schist):*	(HS 10)	—	(HS 9)	(DRA 30, 29)	(DRA 28)	(DRA 27)
(i) Ratio $\dfrac{\text{Combined SiO}_2}{\text{Total R}_2\text{O}_3}$	2·05	—	0·12	0·54	0·46	0·45
(ii) Ratio $\dfrac{\text{Combined SiO}_2}{\text{Total Al}_2\text{O}_3}$	3·73	—	0·21	1·15	1·07	1·16
(iii) Ratio $\dfrac{\text{Total SiO}_2}{\text{Quartz}}$	11·6	—	2·48	1·88	1·55	1·33
(iv) Ratio $\dfrac{\text{Combined SiO}_2}{\text{Combined H}_2\text{O}}$	113·7	—	0·35	2·20	1·93	1·71
(B) *Hope Quarry profile (dolerite):*	(DRA 26)	(DRA 24)	(DRA 25)	(DRA 22, 21)	(DRA 20)	(DRA 19)
(i) Ratio $\dfrac{\text{Combined SiO}_2}{\text{Total R}_2\text{O}_3}$	1·96	0·63	0·63	0·61	0·65	0·56
(ii) Ratio $\dfrac{\text{Combined SiO}_2}{\text{Total Al}_2\text{O}_3}$	3·25	1·21	1·13	1·15	1·22	1·09
(iii) Ratio $\dfrac{\text{Total SiO}_2}{\text{Quartz}}$	512·0	2·60	3·98	4·69	3·54	1·43
(iv) Ratio $\dfrac{\text{Combined SiO}_2}{\text{Combined H}_2\text{O}}$	127·7	3·25	2·69	2·10	2·18	1·34
Intermediate rock.						
(C) *Blue Mountain Quarry profile (quartz diorite):*	(DRA 17)	(DRA 15–11)	(DRA 9)	(DRA 7–3)	(DRA 2)	(DRA 1)
(i) Ratio $\dfrac{\text{Combined SiO}_2}{\text{Total R}_2\text{O}_3}$	0·79	0·54	0·43	0·80	0·86	1·04
(ii) Ratio $\dfrac{\text{Combined SiO}_2}{\text{Total Al}_2\text{O}_3}$	1·18	1·25	0·62	1·30	1·31	5·12
(iii) Ratio $\dfrac{\text{Total SiO}_2}{\text{Quartz}}$	1·48	1·24	1·25	1·29	1·23	1·12
(iv) Ratio $\dfrac{\text{Combined SiO}_2}{\text{Combined H}_2\text{O}}$	32·2	1·56	1·21	2·13	2·14	1·55
Acidic rocks.						
(D) *Mazaruni Quarry profile (granite):*	(HS 7)	(HS 6)	(HS 5)	(HS 4, 3)	(HS 2)	(HS 1)
(i) Ratio $\dfrac{\text{Combined SiO}_2}{\text{Total Al}_2\text{O}_3}$	2·51	1·88	1·37	1·24	1·18	1·20
(ii) Ratio $\dfrac{\text{Combined SiO}_2}{\text{Total Al}_2\text{O}_3}$	2·84	2·16	1·52	1·34	1·24	1·28
(iii) Ratio $\dfrac{\text{Total SiO}_2}{\text{Quartz}}$	2·30	1·79	1·68	1·87	2·06	1·77
(iv) Ratio $\dfrac{\text{Combined SiO}_2}{\text{Combined H}_2\text{O}}$	58·7	19·2	5·48	4·21	3·05	6·34
(E) *Kamwatta Creek profile (gneissose granite):*	(HS 8)		(DRA 35)	(DRA 34)	(DRA 33)	(DRA 32, 31)
(i) Ratio $\dfrac{\text{Combined SiO}_2}{\text{Total R}_2\text{O}_3}$	1·88	—	0·99	1·00	0·93	1·06
(ii) Ratio $\dfrac{\text{Combined SiO}_2}{\text{Total Al}_2\text{O}_3}$	2·14	—	1·13	1·15	1·17	1·45
(iii) Ratio $\dfrac{\text{Total SiO}_2}{\text{Quartz}}$	1·92	—	1·71	1·55	1·26	1·10
(iv) Ratio $\dfrac{\text{Combined SiO}_2}{\text{Combined H}_2\text{O}}$	34·7	—	3·07	3·04	2·96	2·70

results which are usually ascribed to the so-called process of lateritisation. The extent to which this change has proceeded is relatively very great in the basic-rock profiles, but only slight or negligible in the acidic-rock (granite) profiles.

Note. Ratio ii (combined SiO_2/total Al_2O_3) for pure kaolinite theoretically is 1·18; hence ratio values numerically less than 1·18 have been printed in heavy type in Table II, so that here accumulations of alumina over and above that combined in kaolinite (and possibly therefore present in the *free* state) are indicated. Such accumulations appear characteristically in the zones of alteration of basic igneous rocks; they are not generally indicated in the acidic types (granites).

2. Ratio iv (combined SiO_2/combined water) is very much less for the zones of alteration than for the parent rocks, indicating profound hydration during katamorphism.

Note. The combined water ratio for kaolinite is 3·35. In Table II, ratios numerically less than this are printed in heavy type, indicating degrees of hydration greater than may be ascribed merely to the formation of this ideal secondary mineral, and suggesting that other hydrated minerals, such as hydrous iron oxides, largely contribute to the high hydration values. In general, hydration seems to be greatest in the altered products of the more basic rocks containing the largest amounts of iron oxide.

(ii) *Zone of secondary changes.*

Silica ratios (Nos. i, ii) for the zones of secondary changes in the basic and intermediate rock profiles are higher than those for the zones of (completed) alteration, but in the acidic rock profiles they tend to be slightly less. These features suggest that *resilication* has proceeded in the more basic rock profiles, and has thus produced a general similarity in the composition of all the secondary katamorphic materials, whether derived from basic and intermediate, or from acid rocks. The importance and significance of this "equalising process" is specifically stressed by Harrison in the final summary of his findings, in the following words of his monograph.

(1) "Under tropical conditions, the katamorphism of *basic and intermediate* rocks, at or close to the water table, under conditions of more or less perfect drainage, is accompanied by the almost complete removal of silica and of calcium, magnesium, potassium and sodium oxides, leaving an earthy residuum of aluminium trihydrate (in its crystalline form of gibbsite), limonite, a few unaltered fragments of felspars, in

some cases, secondary quartz, and the various resistant minerals originally present in the rock. This residuum is termed *Primary Laterite.*"

(2) "The process of primary lateritisation is succeeded by one of *resilication,* gradually resulting in the vast masses of lateritic earths or argillaceous laterite, which, in the tropics, so frequently cover up wide areas of basic and intermediate rocks."

(3) "Under tropical conditions, *acidic rocks* do *not* undergo primary lateritisation, but gradually change through katamorphism into pipe or pot clays, or more or less quartziferous and impure kaolins[1]."

It will be noted that Harrison specifies the *conditions of drainage* in describing tropical katamorphism of igneous rocks. Throughout his monograph, he differentiates between good and bad drainage conditions in their relation to the processes of "primary lateritisation" and of "resilication[2]." He adduces evidence, based on rainfall and evaporation records, that demonstrates widely divergent water relations in the various localities in British Guiana where he examined the products of igneous rock katamorphism. His main conclusions are expressed in the following words:

"The primary process of formation of laterite is the same on high or on low levels. The after-processes differ materially. On *well-drained mountain plateaux,* where the rainfall is very high (*i.e.* above 150 inches), and more or less continuous throughout the year, primary laterite appears to be permanent as such, and where not exposed to washing, accumulates in considerable thickness."

On badly drained low-lying areas, on the other hand, primary laterite appears not to be permanent, but to give rise (according to Harrison) to argillaceous earths, formed through resilication of the gibbsite component of the laterite by ascending ground water containing silica or silicates in solution.

The differences between the final katamorphic products formed at badly drained low levels, as contrasted with those formed at well-drained high levels, are ascribed by Harrison to "the variations in the facilities

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[1] This difference between the primary katamorphism of basic and intermediate rocks and of acidic rocks in the tropics has also been recently stressed by J. B. Scrivenor (10, 11), who states that: "In Malaya, there is no doubt that the main product of weathering in acid igneous rocks is a hydrated silicate of aluminium. On the other hand, over basic rocks, aluminium hydrate is a general weathering product."

[2] Harrison's views regarding the significance of water relations appear to coincide with those expressed by Morrow Campbell (1), who differentiates between "alteration," which proceeds in the "zone of permanent saturation," and "weathering" which occurs within the "zone of intermittent saturation," and distinguishes both from the changes proceeding in the "zone of non-saturation," above the reach of "vadose water."

for draining off the water from the surface of the lateritising rock, which (on high mountain plateaux) are perfect, whilst (on low-lying plains) the ground water at or below the water table is always more or less sluggish or practically stagnant."

"During the passage upwards by capillary attraction in dry seasons, when the evaporation exceeds the rainfall, the silica-bearing water derived from the underlying rock surface forms surface films of moisture in the spongy primary laterite where some of the silica reacts with some of the finely divided gibbsite to form a hydrated aluminium silicate, principally a crystalline kaolin. During this action, another portion of the aluminium trihydrate is taken into solution and is deposited from the ascending water as films, veins, spheroidal and other masses of gibbsite."

Water relations at the different profile sites. According to Harrison's meteorological records (including data referring to evaporation of water from tanks) the five particular sites investigated by us fall into two main categories as regards water relations. Sites C, B and D (Blue Mountain, Hope and Mazaruni) belong to one category, and sites A and E (Tiger Hill and Kamwatta Creek) to the other. The first three (river side) sites lie within the undulating, low-lying plains region, where drainage conditions may be relatively poor, the water table in the wet season being generally high. The other sites lie within the hilly North-West District (10), where drainage conditions are probably better. The yearly rainfall at each site is almost identical, namely, about 106 inches, of which some 93 inches fall in eight rainy months, and the rest, 13 inches, in four dry months. The main difference between the two regions, however, lies in the fact that within the first region evaporation during the dry season is about double the amount of the rainfall, whereas in the North-West District it barely equals the rainfall. These conditions should favour resilication, but more in the first region than in the second. The tabulated chemical data appear to be in accordance with this generalisation.

It is nevertheless evident that detailed studies of katamorphism ought to include periodic measurements of fluctuations in ground-water levels. Topographical observations alone are not sufficient to describe precisely the water relations within particular areas where characteristic soil profiles are being developed.

(iii) *Zone of leaching.*

1. Silica/sesquioxide ratios (Nos. i and ii) for the topsoil in the case of basic rock types are numerically about the same as those for the zones

of secondary changes. In the case of the intermediate and the acidic rock types, however, the ratio values tend to rise, indicating loss of sesquioxides through leaching (podsolisation).

2. Combined SiO_2/quartz ratios (No. iii) in every case show steady decreases between the zones of alteration and the surface soils, indicating accumulation of resistant quartz, and implying that the chief process affecting the topsoil is a mechanical washing action by water whereby finely divided kaolin is transported to lower layers.

3. Combined SiO_2/H_2O ratios (No. iv) show continued marked decreases, indicating further hydration of siliceous minerals in the surface material.

The most striking feature revealed by the data is the general similarity in composition of the topsoil layer of each of the five profiles. This may be regarded as a further expression of the equalising process initiated in the zone of secondary changes, whereby the alteration products of the basic rock types are resilicated, and thus eventually come to resemble the relatively slightly modified alteration products of the acidic rock types[1].

5. Conclusions from silica contents and alizarin adsorption data.

It is evident from our own results that sufficiently precise and definite information regarding the characteristic transformations which occur during tropical katamorphism of igneous rocks may nevertheless be acquired by simpler and more rapid means than the laborious petrographical and conventional chemical methods such as were applied by Harrison in his studies in British Guiana. Our experience suggests that the application of the alizarin adsorption procedure, accompanied by determinations of free and combined silica, furnish sufficient evidence

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[1] Since this article was written, the writers have noted the observations recorded by Dr J. A. Prescott in a letter to *Nature*, Nov. 8, 1930, p. 724, wherein he stresses the similarity between leached tropical soils and temperate savannah soils occurring in Australia, both of which are apparently podsolic in character.

"Primary laterite" may perhaps best be regarded as a *parent material* from which different soil profiles may be developed through the operation of different climatic factors, especially those that control water relations. According to this view, the geological origin and age of the parent laterite become matters of little moment to the soil investigator. Whether the development of the soil profile be "consequent" on the formation of laterite (as appears to be the case in British Guiana), or whether it follows at a much later period ("subsequent"), when the climate may have changed, are questions that mainly concern the geologist.

Table III. *Summarised results for silica contents and alizarin values.*

	Parent rock	Zone of alteration		Zone of secondary changes		Zone of leaching Topsoil
		Commencing	Completed		Transition	

Basic rocks.

(A) *Tiger Hill profile (hornblende schist)*:

	(HS 10)	—	(HS 9)	(DRA 30, 29)	(DRA 28)	(DRA 27)
Total silica	49·8	—	12·4	45·1	48·0	54·1
Quartz (%)	8·6	—	40·3	53·2	64·6	75·1
Combined silica (%)	91·4	—	59·7	46·8	35·4	24·9
Alizarin value (1)	—	—	(? high)	19	24	26
" " (2)	—	—	—	96	99	82
Ratio $\dfrac{\text{Combined } SiO_2}{\text{Free } Al_2O_3}$	∞	—	—	7·6	4·7	3·5
Ratio $\dfrac{\text{Combined } SiO_2}{\text{Active } Fe_2O_3}$	∞	—	—	3·5	2·6	2·5

(B) *Hope Quarry profile (dolerite)*:

	(DRA 26)	(DRA 24)	(DRA 25)	(DRA 22, 21)	(DRA 20)	(DRA 19)
Total silica	51·2	38·0	37·0	35·1	40·7	55·9
Quartz (%)	0·2	38·4	25·1	16·0	28·2	69·7
Combined silica (%)	99·8	61·6	74·9	84·0	71·8	30·3
Alizarin value (1)	3	11	17	44	34	16
" " (2)	9	38	65	105	101	61
Ratio $\dfrac{\text{Combined } SiO_2}{\text{Free } Al_2O_3}$	102·2	13·6	9·1	4·5	5·5	7·0
Ratio $\dfrac{\text{Combined } SiO_2}{\text{Active } Fe_2O_3}$	85·2	9·3	5·5	4·2	7·0	7·0

Intermediate rock.

(C) *Blue Mountain Quarry profile (quartz diorite)*:

	(DRA 17)	(DRA 15–11)	(DRA 9)	(DRA 7–3)	(DRA 2)	(DRA 1)
Total silica	59·5	63·3	58·9	71·7	75·4	83·3
Quartz (%)	67·6	80·6	80·1	77·7	81·5	89·6
Combined silica (%)	32·4	19·4	19·9	22·3	18·5	10·4
Alizarin value (1)	6	31	49	26	21	15
" " (2)	10	64	107	84	56	5
Ratio $\dfrac{\text{Combined } SiO_2}{\text{Free } Al_2O_3}$	21·4	2·6	1·6	4·1	4·5	3·8
Ratio $\dfrac{\text{Combined } SiO_2}{\text{Active } Fe_2O_3}$	48·3	4·9	1·6	3·1	6·3	43·5

Acidic rocks.

(D) *Mazaruni Quarry profile (granite)*:

	(HS 7)	(HS 6)	(HS 5)	(HS 4–3)	(HS 2)	(HS 1)
Total silica	72·8	73·9	70·2	65·0	61·1	68·7
Quartz (%)	43·5	55·7	59·4	53·4	48·6	56·6
Combined silica (%)	56·5	44·3	40·6	46·6	51·4	43·4
Alizarin value (1)	—	—	0	0	—	—
" " (2)	—	—	10	10	—	—
Ratio $\dfrac{\text{Combined } SiO_2}{\text{Free } Al_2O_3}$	—	—	Very large	Very large	—	—
Ratio $\dfrac{\text{Combined } SiO_2}{\text{Active } Fe_2O_3}$	—	—	29·4	29·4	—	—

(E) *Kamwatta Creek profile (gneissose granite)*:

	(HS 8)	—	(DRA 35)	(DRA 34)	(DRA 33)	(DRA 32–31)
Total silica	72·5	—	63·7	66·9	75·9	86·9
Quartz (%)	52·1	—	58·6	64·6	79·3	90·5
Combined silica (%)	47·9	—	41·4	35·5	20·3	9·5
Alizarin value (1)	—	—	3	4	3	5
" " (2)	—	—	77	72	5·3	24
Ratio $\dfrac{\text{Combined } SiO_2}{\text{Free } Al_2O_3}$	—	—	52·8	39·5	31·4	9·7
Ratio $\dfrac{\text{Combined } SiO_2}{\text{Active } Fe_2O_3}$	—	—	8·5	8·2	7·5	8·3

of the nature of these changes without recourse to more elaborate methods of analysis.

In support of this contention we have collected together in Table III the numerical results obtained by these means, and which appear to be adequate for the purpose of characterising the different zones displayed in soil profiles. An important requisite in the advocated procedure, however, is that sufficiently minute and detailed sampling, such as that employed by Harrison, should throughout be followed, particularly in the neighbourhood of the parent rock, otherwise the more important features of the katamorphic processes may be completely overlooked. Unfortunately, the significance of these details of sampling procedure was not fully appreciated by us at the time when our field collections were made, so that our data are not sufficient in all cases to reveal the different features which the various profiles present, particularly in the immediate vicinity of the parent rocks.

The silica and alizarin adsorption data contained in Table III demonstrate the following characteristic features in the different profiles:

(A) *Zones of alteration.*

1. The extensive liberation and removal of free silica from the original silicate minerals of the more basic rocks ("lateritisation"), but not from those of the acidic rocks.

2. The concomitant accumulation of free sesquioxides as gibbsitic alumina and hydrous red iron oxides[1] in the basic rock profiles ("lateritisation").

(B) *Zones of secondary changes.*

1. Fixation of silica, partly through resilication of free alumina in the more basic rock profiles, and partly through the formation of secondary crystalline quartz.

2. Further accumulation of hydrous red iron oxides in the more basic rock profiles.

(C) *Zones of leaching.*

1. Marked accumulations of quartz in all the profiles.

2. Slight diminution of free alumina ("podsolisation"), except in the most basic rock (hornblende schist) profile.

3. Marked diminution of hydrous iron oxides, particularly in the intermediate and acidic rock profiles ("podsolisation").

4. A general similarity in the composition of the topsoil of each

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[1] Alizarin values for hydrous iron oxides; see Appendix B.

profile, excepting that the more basic types contain the greatest surplus amount of residuary free sesquioxides.

PART II. ECOLOGICAL ASPECTS.

Considered from the points of view of vegetation relationships and suitability to agricultural utilisation, the five profile sites appear to be essentially similar. At each site, the vegetation chiefly consists of low forest of "mixed" composition, comprising secondary growths following partial clearings of the primary forest by aboriginal Indians or by immigrant settlers. The regenerated flora contains the characteristic member, *Cecropia peltata* (Congo Pumpwood or Trumpet Tree), which is widespread throughout the American tropics. In the low-lying plains area (where sites C, B and D occur), few traces of the original forest flora remain. Here the regenerated bush is represented by *Cecropia*, in association with *Maxmilliana regia* (Cocorite Palm), *Pentaclethra filamentosa* (Trysil), *Myristica surinamensis*, *Inga* spp. and various species of Melastomaceae and Bignoniaceae.

In the North-West District (where sites A and E occur), the vegetation occupying the sandhills (derived from acidic gneissose rocks) closely resembles that of the sandy red earths (derived from basic schists). In places, original "mixed" forest still prevails. According to a recent report by the British Guiana Department of Forestry, the principal members comprising this original flora are: *Diplotropis* sp., *Protium decandrum*, *Goupia glabra*, *Mimusops globosa* (Balata), *M. elata*, *Aspidosperma excelsum*, *Catostemma* sp., *Carapa guianensis* (Crappo or Crabwood), *Sterculia excelsa*, and various species of Lecythidaceae. There is little undergrowth. Where the original forest has been cleared, secondary low forest, characterised by *Cecropia*, *Vismia macrophylla* (Bloodwood), *Inga* spp. and *Sloanea* spp., appears.

In order the more clearly to demonstrate the essential similarity between the soils occurring at the five sites, certain soil constants, commonly determined in profile studies at the Imperial College of Tropical Agriculture, have been measured by methods elsewhere described (3). The results obtained are presented in Table IV.

The data indicate the following main features:

1. The *topsoil* in every case is sandy, and much lighter in texture than the subsoil, especially in the intermediate and acidic (quartzose) rock profiles. Its reaction is acidic, becoming less acid in the subsoil. The reaction of the material comprising the zone of alteration is neutral or alkaline in the basic rock profiles, but acidic in the others.

Table IV. *Soil constants for the different profile-samples.*

Profile	Lower depth (in.)	Sand content (%)	Index of texture*	Reaction Normal (pH)	Reaction Ex-change (pH)	Organic matter content (%)	Nitrogen content (%)	C/N ratio	Easily available nutrients solution (mhos ×10⁻⁶)	Rate of solution	Available K₂O 1% citric sol. Parts per 10,000 of material	Available P₂O₅ 1% citric sol.
(A) Tiger Hill profile (hornblende schist):												
DRA 27. Red-brown humic clay	9	48	28	6·2	5·5	1·82	0·16	6·5	105	41	1·24	0·00
28. Tawny red gravelly clay	27	38	35	6·6	6·2	1·19	0·09	7·6	90	0	0·72	0·06
29. Do.	45	40	38	6·6	5·8	0·71	0·05	8·6	54	1	0·61	0·00
30. Do.	63	40	44	6·8	5·7	0·37	0·04	5·6	59	0	0·51	0·00
(B) Hope Quarry profile (dolerite):												
DRA 19. Red-brown topsoil	21	39	28	4·9	4·4	2·44	0·13	10·5	29	7	0·24	0·07
20. Red earth	43	21	40	5·0	4·4	0·68	0·04	9·3	9	2	0·17	Nil
21. Do.	88	24	39	5·1	4·5	0·78	0·04	10·7	12	1	0·58	„
22. Red mottled earth	106	32	41	5·3	5·0	0·32	0·03	6·0	6	1	0·15	„
23. Tawny yellow earth	110	60	13	7·2	6·3	0·11	0·01	7·1	10	4	0·58	0·00
24. Pale yellow earth	112	73	10	7·0	6·7	0·10	0·01	7·5	34	4	0·25	0·08
26. Parent rock	—	—	—	7·1	6·8	—	—	—	84	78	—	—
(C) Blue Mountain Quarry profile (quartz diorite):												
DRA 1. Dark grey topsoil	17	77	5	4·7	4·3	1·20	0·08	9·0	39	12	0·52	0·20
2. Pale brown subsoil	29	69	9	4·6	4·1	0·40	0·03	7·0	14	4	0·38	0·10
3. Transition	35	61	14	4·9	4·3	0·41	0·03	7·7	18	3	0·46	0·00
4. Red earth	47	63	14	4·9	4·4	0·26	0·03	5·5	7	3	0·39	0·00
5-7. Orange-red earth	60	63	15	5·1	4·5	0·18	0·02	4·5	8	2	0·12	0·06
8. Ochreous earth	62½	62	14	5·2	4·6	0·31	0·03	6·4	10	1	0·58	0·00
10. Hard laterite	63	—	—	5·8	5·6	0·51	0·03	10·7	13	2	—	—
11. Rotten rock	64¼	—	—	5·3	4·5	0·45	0·04	7·2	14	2	0·31	0·23
12-15. Do.	71¼	—	—	5·4	4·8	0·48	0·04	6·8	8	4	0·12	0·15
17. Parent rock	—	—	—	7·7	7·6	—	—	—	98	91	—	—
(D) Mazaruni Quarry profile (granite):												
DRA 56. Granite sand	—	65	12	5·5	5·0	0·21	0·02	5·2	170	27	0·06	0·07
(E) Kamwaita Creek profile (gneissose granite):												
DRA 31. Sepia sand	2	90	8	4·5	3·9	2·87	0·14	12·2	101	24	0·11	0·83
32. Grey sand	20	80	10	4·5	4·2	1·85	0·08	13·0	88	16	0·45	0·12
33. Pale brown sandy clay	23	68	19	4·7	4·2	0·06	0·06	9·3	104	0	0·29	0·11
34. Brownish buff clay	28	48	32	4·6	4·1	—	—	—	—	—	0·19	0·09
35. Orange-pink clay	76	43	31	4·8	4·2	—	—	—	25	0	0·06	0·18

* Moisture content at sticky point *minus* ⅓ sand content.

2. *Organic matter* penetration is somewhat deep, but is mainly confined to the topsoil layer in which most of the absorbing roots occur. Its carbon/nitrogen ratio fluctuates considerably, but tends to diminish in the lower profile horizons. The average value of this ratio lies between 7·0 and 10·0, indicating considerable decomposition of plant residues.

3. The *nutrient status* of the soils is poor, both easily available nutrients (including citric-soluble potash and phosphate) and rates of solution of potential nutrients being exceptionally low in amount, even in the deeper partially altered rock material. On the whole, the North-West District soils appear to be the better, a difference which may be attributed to the fact that the original forest conditions there have been least affected by exploitation, so that the natural surface humic layer, derived from plant residues, is still intact.

SUMMARY.

1. Five typical British Guiana soil profiles, derived under humid tropical conditions from igneous rocks of different silica contents, are described, and the results of their detailed petrographical, chemical and ecological examination are presented and discussed.

2. Each kind of rock has produced a sandy, acidic, nutrient-deficient surface soil, but the katamorphic changes that have occurred during rock alteration, weathering and leaching have been fundamentally different.

3. The *basic* and *intermediate* igneous rocks have given rise within the zone of alteration to "primary gibbsitic laterite," covering the parent rock in a thin crusty layer. Within the zone of secondary changes, the laterite has been resilicated, presumably through the agency of ground water containing soluble silica or alkali silicates (partially in the more basic rock profiles, and completely in the intermediate types), into kaolinitic earths, coloured yellow and red by hydrous iron oxides. Residuary gibbsite and iron oxides have accumulated in the more basic profiles as concretionary gravelly masses.

4. The *acidic* igneous rocks, on the other hand, have given rise *directly* to kaolinitic earths, only slightly coloured by hydrous iron oxides. Liberated silica has here accumulated as secondary quartz.

5. These changes may be satisfactorily demonstrated by simple determinations of free and combined silica, and by the application of an alizarin adsorption method for estimating free gibbsitic alumina and active hydrous iron oxides, without recourse to detailed petrographical and chemical analyses. Evidence is adduced in support of this contention.

6. The significance of the effects of fluctuating ground water in the development of humid tropical soil profiles is especially stressed.

7. Reference throughout is made to results obtained by the late Sir John Harrison, contained mainly in an unpublished manuscript, to which access was afforded by the courtesy of the Government of British Guiana.

8. The investigation has revealed the origin, genesis and relationships of tropical red earths and leached sandy earths as developed in British Guiana and in the neighbouring regions of South America.

APPENDIX A.

TRI-ACID-DIGESTION METHOD FOR DETERMINING COMBINED SILICA AND QUARTZ IN LATERITES, BAUXITES, LATERITIC CLAYS AND RED EARTHS.

Government Laboratory, British Guiana.

One gm. of material, ground to impalpable powder, is treated in a 100 c.c. silica dish with 30 c.c. of a mixture of conc. sulphuric acid (sp. gr. 1·84, four parts), conc. hydrochloric acid (sp. gr. 1·19, two parts) and conc. nitric acid (sp. gr. 1·40, one part). The basin is covered by a watch-glass, and its contents gently heated on a sandbath until brown fumes cease to be evolved. The cover is removed and the mixture strongly heated until the evolution of white fumes ceases. The paste is allowed to cool, and 50 c.c. of 10 per cent. hydrochloric acid are added, and the whole heated to the boiling point, and filtered through a Whatman 42 paper. The whole silica precipitate is washed and ignited in a platinum crucible. It is warmed with 25 c.c. of 2 per cent. caustic soda, with occasional addition of water, for three hours, during which "combined" silica, liberated by the tri-acid treatment, is dissolved, whilst quartz remains unattacked. The quartz is separated and washed on a Whatman 42 filter paper, and the filtrate is evaporated to dryness and heated at 130° C. for one hour to effect silica dehydration. Fifty c.c. of 10 per cent. hydrochloric acid are next added, and the mixture brought to boiling point. The precipitate is filtered through a Whatman 40 paper. True silica contents of the quartz and the combined silica components are separately determined by weighing the ignited residues before and after treatment with hydrofluoric acid. The residues are fused with potassium hydrogen sulphate and added to the main filtrate from the whole silica precipitate for the determination of sesquioxides, alkali and alkaline earth metals.

Preparation of tri-acid mixture. The procedure followed in mixing the three concentrated acids largely decides the reactivity of the reagent. The best method is as follows. A large resistance-glass flask containing the hydrochloric acid is immersed in cold water and the sulphuric acid added to it drop by drop with frequent shaking. The nitric acid is subsequently added in similar manner. Sufficient tri-acid mixture should be made for a small number of determinations only; it is inadvisable to prepare a large stock of the reagent.

APPENDIX B.

ALIZARIN ADSORPTION BY IRON OXIDE MINERALS.

In the previous chapter of this series[4], results were presented (Table I) showing the magnitudes of the adsorption of alizarin by various artificial preparations of hydrous ferric oxides, prepared according to directions given by Morrow Campbell[1]. The values, per gram Fe_2O_3, for the fresh air-dried specimens varied between 1500 units for the lesser hydrated types (average ignition loss 13·7 per cent.) and 2500 units for the more heavily hydrated types (ignition loss 26·5 per cent.).

We have since procured various specimens of authentic iron oxide minerals, and have determined their contents of combined water and ferric oxide, and their alizarin adsorption values. The results obtained are set out in the following table:

Results of alizarin tests on iron oxide minerals.

				Alizarin values (units per gm. of material)						
		Composition		Fresh, unignited material				Ignited material		
Mineral		Loss on ignition (%)	Fe₂O₃ content (oven dry)	Material	Oxalate extract	Difference	Corrected (per gm. anhydrous Fe₂O₃)	Material	Oxalate extract	Difference
Name	Colour									
Turgite (G)	Yellow	17·0	78·8	182	67	115	146	121	87	34
Limonite (EA)	Yellow	10·8	81·8	49	20	29	35	23	9	14
,, (G)	Yellow	10·3	76·4	48	20	28	36	29	11	18
,, (T)	Orange	10·5	82·9	51	21	30	36	34	18	16
Goethite (EA)	Crimson	4·7	91·1	48	44	4	4	18	7	11
,, (G)	Crimson	1·2	95·8	41	38	3	3	15	10	5
Haematite (G)	Bright red	1·0	97·9	55	56	0	0	20	18	2
,, (T)	Red-brown	0·3	86·0	79	79	0	0	16	12	4
Surficial Ironstone (BG)		10·4	51·9	67	24	43	83	26	7	19
Purple-red iron oxide (BG)		7·6	65·4	80	27	53	81	56	13	43

(G) Specimens obtained from Messrs Gregory, London.
(EA) Specimens obtained from Messrs Eimer and Amend, New York.
(T) Trinidad specimens.
(BG) British Guiana specimens.

Discussion. In the table, the minerals are arranged in order of increasing contents of combined water. The corrected alizarin values, per gram anhydrous ferric oxide, alone need here be considered. The turgite specimen, containing the highest amount of combined water (17 per cent.), gave the highest alizarin value (146 units), which is, however, about 10 times smaller than that given by the less hydrated *artificial* hydrous ferric oxide preparations. Limonite (average combined water content 10·5 per cent.) gave alizarin values of about 36 units; goethite (of still lower degree of hydration), gave values of 4 units only, but haematite showed no appreciable adsorption of alizarin.

It is evident from these results that the *calculating* factors employed in our previous investigation [4], namely 0·066 and 0·040, are very much too low. For *turgite*, the factor yielded by the recent results is 0·68 gm. anhydrous Fe_2O_3 per alizarin unit, for *limonite* 2·77, and for *goethite* 25·0. Since *haematite* shows little or no alizarin adsorption, its presence in earthy materials cannot be detected nor estimated by the suggested procedure.

Applying these conclusions to the results obtained with the various soil and subsoil samples described in the previous paper, it is evident that the free hydrous iron oxide component of practically all the samples examined is more reactive (more highly adsorptive of alizarin) than the mineral turgite, and very much more reactive than the minerals limonite, goethite and haematite. On the other hand, it is less reactive than any of the artificial preparations of hydrous ferric oxide. These conclusions imply that the free hydrous iron oxide component of clays and soils generally includes a hydrated form of high reactivity, and that attempts to assign definite mineral formulae to this component are unwarranted.

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THE AVAILABILITY OF MANGANESE
IN THE SOIL.

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(With Three Text-figures.)

I. INTRODUCTION.

DURING the course of some previous experimental work on the essential nature of manganese for plants (Samuel and Piper, 1929) the results of certain pot experiments with soil deficient in available manganese suggested that there were factors controlling the availability of this element other than the reaction of the soil. Owing to the ease with which manganese dioxide is reduced to the manganous state under certain conditions, and the much greater solubility of manganous compounds than those of manganese dioxide, it was suspected that the oxidation-reduction equilibrium within the soil would prove to be an important factor. In the present paper the availability of manganese is discussed and further experimental evidence is adduced to show that it is influenced by at least two factors, soil reaction and the oxidation-reduction equilibrium acting in intimate association.

Manganese is one of those elements which is required in traces only for normal plant growth, and if these small quantities are not available growth is limited and characteristic disease symptoms appear on the plants. Instances of diseases, which are now known to be particular cases of manganese deficiency, have been reported by Arrhenius (1924), Gilbert, McLean and Hardin (1926), Schreiner and Dawson (1927), Willis (1928), and Ogg and Dow (1928) among others. Grey speck disease of oats (*Dörrfleckenkrankheit*) has been shown to be such a disease (Samuel and Piper, 1928).

Although manganese is an essential element, it has a toxic effect on plant growth if present in the soil solution in excessive amounts. It appears from Brenchley's work (1927) that 1 part of manganese in 40,000 parts of solution produced a definite toxic effect on barley in water cultures, and 1 part in 400,000 was toxic to peas. The limits of concentration are probably wider in soils than in water cultures. Between the

limits that would cause either deficiency on the one hand or toxicity on the other, plants make normal growth, and the supply of available manganese is reflected in their manganese content. In South Australia the manganese content of oat plants at the flowering stage has been found to vary from about 10 p.p.m.[1] to 290 p.p.m., nearly a thirtyfold difference. Considerable variations have been found here for other plants. Similar results, due to varying soil conditions, have been reported elsewhere by Peterson and Lindow (1928) and Davidson (1929). Gilbert, McLean and Hardin (1926) found the manganese content of spinach to range from 23 to 103 p.p.m. and Remington and Shiver (1930), examining another twelve samples, found the variation to be from 52 to 253 p.p.m.

Such a very wide variation is not found in the case of the major essential elements such as potassium or calcium. From the large number of analyses collected by E. von Wolff, Tollens (1901) quotes figures for several species of plants, and the widest variation was eightfold for potassium in meadow hay and ninefold for calcium in wheat. Manuring with potash or lime has little relative effect on the amounts present in the ash, very heavy applications of fertiliser being required to double the percentages found.

Little is as yet definitely known concerning the function of manganese in the plant. McHargue (1922) claimed that it is connected with carbon assimilation, and in this connection the high concentration of manganese in the leaves and the lower concentrations in the roots of various plants is interesting. Remington and Shiver (1930), in examining a number of different vegetables (beets, carrots and turnips), found from three to eight times as much manganese in the leafy parts as in the roots.

II. EXPERIMENTAL.

Up to the present, manganese-deficiency diseases have been found to occur in Australia on four very distinctive soil types, and the opportunity has been taken of studying three of these in detail. Since oats growing on these particular soils normally exhibit typical symptoms of manganese deficiency, this plant has been used as an indicator of the availability of manganese in the pot experiments to be described. Changes in the availability are reflected in the severity or absence of disease symptoms, the amount of growth, and also in the manganese content of the plants.

[1] p.p.m. = parts of manganese (Mn) per million parts of dry matter.

Of the three soils used for the experimental work that from Mt Gambier is a light, open-textured, dark brown soil derived from the ash of a Pleistocene volcanic formation. It is slightly alkaline in reaction (pH 7·4). Although it contains manganese equivalent to 0·045 per cent. Mn_3O_4 this appears to be unavailable under certain conditions. The Penola soil is a heavy black clay (60 per cent. clay) of a very different type. It is nearly neutral in reaction (pH 6·9) and has been formed under swampy conditions, which probably partly accounts for its very low content of total manganese (0·007 per cent. Mn_3O_4). The soil from Corney Point, Yorke Peninsula, is a light, open-textured, grey coloured soil typical of a coastal plain formation. It is highly calcareous, containing nearly 60 per cent. of calcium carbonate, and its reaction is pH 8·0. It contains manganese equivalent to 0·026 per cent. Mn_3O_4.

The fourth Australian example occurs on the mallee (*Eucalyptus astringens*) soils of Western Australia, soils associated with the breakdown of lateritic conglomerate ridges. No detailed experimental work has so far been carried out on this soil.

For comparison with the first three soils, Glen Osmond soil from the experimental field of the Waite Institute has been used. This soil is a brown-red, fine, sandy loam (pH 7·4), normally carrying healthy oats. No sign of manganese deficiency has ever been observed in crops growing on it.

Manganese has been determined throughout by the colorimetric-periodate method. It was found that repeated extractions with hydrochloric acid did not always dissolve the whole of the manganese in the ash of many plants, even when ashed at low temperatures. This was particularly the case where there was a large proportion of silica in the ash. In these instances the ash was first extracted with hydrochloric or nitric acid and the residue re-ashed and digested in a platinum dish with a few drops of sulphuric acid and sufficient hydrofluoric acid to volatilise the silica. The solution was then evaporated until almost dry, and the residue taken up in acid, filtered and added to the first filtrate. In most of the ash analyses the permanganate colour was developed in the presence of phosphoric acid instead of sulphuric acid. By using phosphoric acid, the precipitation of calcium sulphate was avoided.

A. *Laboratory tests with soils.*

To determine the effect of reducing conditions on the solubility of manganese in alkaline soils 300 gm. portions of Mt Gambier, Penola, and Glen Osmond soils were waterlogged in beakers by the addition of

600 ml. of water and stored in the laboratory for a period of three weeks. Similar quantities of the three soils were mixed with 0·25 per cent. of their weight of dextrose and waterlogged. Two days after starting the experiment the contents of all beakers were stirred and a pronounced smell, suggestive of butyric and lactic fermentation, was noticed in those soils to which dextrose had been added. About a week later the supernatant liquid of those beakers containing dextrose was noticeably more grey-black (ferrous sulphide) than that of the other beakers. All soils now had a foul odour. At the end of the experiment water was added to bring the soil : water ratio to 1 : 5, and after shaking for one hour, the solutions were filtered through candle filters and water-soluble manganese determined.

At the same time as the above further lots of the Mt Gambier and Glen Osmond soils were waterlogged both with and without the addition of 0·25 per cent. of dextrose. Instead of leaving them undisturbed, air was bubbled through the mixture for half an hour twice daily over the same period in order to make conditions less anaerobic than in the former. Solutions from these were also filtered and the water-soluble manganese determined as before. The results for both experiments are given in Table I.

Table I. *Showing the amounts of water-soluble manganese produced in soils by waterlogging.*

	Mt Gambier soil Mn (p.p.m.)*	Pinola soil Mn (p.p.m.)*	Glen Osmond soil Mn (p.p.m.)*
Soil waterlogged	1·19	0·08	6·3
Soil + 0·25 % dextrose waterlogged ...	5·45.	0·32	13·0
Soil waterlogged in current of air ...	0·05	—	3·0
Soil + dextrose waterlogged in current of air	2·38	—	4·8

* Results given as parts of manganese per million parts of dry soil.

From the table it will be seen that the addition of dextrose to the soils before waterlogging was always accompanied by a large increase in the amount of water-soluble manganese. This was associated with the greater bacterial activity in these soils, anaerobic conditions developing sooner and becoming more pronounced than in the soils to which no dextrose had been added. Such reducing conditions are very favourable to the reduction of manganese dioxide to some   manganous compounds. In those cases in which air was bubbled th   amount of water-soluble manganese was signific with the fact that although anaerobic co

decr eased their intensity and conditions for reduction were not so favou rable, correspondingly smaller amounts of soluble manganese appea ring. The decrease from 1·19 to 0·05 p.p.m. in the Mt Gambier soil is particularly noticeable. Under fully aerobic conditions a trace only o. r even no water-soluble manganese could be detected in these soils.

A fu rther instance of the solubility of manganese following water-logging w ras observed at the end of the pot experiments when one of the pots cont. aining Corney Point soil became accidentally waterlogged for a few days . This pot was drained and the drainage water collected. After filtering th rough collodion, soluble manganese was determined in the filtrate and ι 0·6 p.p.m. was found. No soluble manganese could be detected in the soil ι before waterlogging. Manganese is evidently reduced and rendered solu ible in waterlogged soils even as alkaline as this latter (*p*H 8·0).

Further ex, periments were planned to determine the changes occur-ring on the add ition of a soluble manganese salt to the soil. Two lots of 100 gm. each of Mt Gambier and Glen Osmond soils, to which 95 p.p.m. of manganese (a. ʒ manganese sulphate) had been added, were stored at 45 per cent. satu ration for three weeks and the manganese soluble in a 1 : 5 water ext ract was then determined and compared with that obtained from sim. ilar lots of the soils plus manganese sulphate which had been stored at ! 90 per cent. saturation. No water-soluble manganese was found in either soil stored at 45 per cent. saturation, but the Mt Gambier soil at 90 p er cent. saturation contained the equivalent of 2·7 parts of manganese p er million parts of soil, and the Glen Osmond soil showed the presence of 0·76 p.p.m. Thus the soluble manganese added to both soils was whol ly converted to a form insoluble in water in the soils kept at 45 per cent. saturation and almost wholly so in the soils at 90 per cent. saturation.

The nature of this ins oluble form was determined in a further experi-ment. Similar lots of soil with and without the addition of 95 p.p.m. of manganese, as mangan ese sulphate, were stored for four weeks at 45 per cent. saturation. T hey were then shaken for 30 minutes with 0·05 *N* sulphuric acid, rapic lly filtered and extracted with repeated ad-ditions of acid until one li tre of filtrate had been collected. Dilute sulphuric acid of thi   h was used, as it has no solvent effect on manganese   nite sufficient to displace all manganese   m. Following this extraction the soil was   0·05 *N* sulphuric acid containing 0·2 per

cent. hydroquinone, this dissolving manganese dioxide, manganous sulphate being formed. The experimental results are given in Table II.

Table II. *Showing the amounts of replaceable manganese and manganese dioxide in two soils kept at 45 per cent. saturation, and the conversion to manganese dioxide of soluble manganese compounds added to these soils.*

	Glen Osmond soil (p.p.m.)	Glen Osmond soil + 95 p.p.m. Mn	Mt Gambier soil (p.p.m.)	Mt Gambier soil + 95 p.p.m. Mn
Manganese as				
replaceable manganese	65·7	83·7	4·2	20·9
manganese dioxide	315·0	378·7	124·0	206·7

From the above table it will be seen that in each case the added manganese was converted largely to manganese dioxide, approximately four-fifths appearing in this form and the remainder as replaceable manganese. No determinations were made at different time intervals, but from other work it would appear that the oxidation is progressive and, given a longer period, the conversion to manganese dioxide would have been even greater. The very low value for replaceable manganese in the Mt Gambier soil (4·2 p.p.m.) compared with that in the Glen Osmond soil (65·7 p.p.m.) is also noticeable.

B. *Pot experiments.*

To determine the influence of soil reaction on the availability of manganese, two series of pot experiments were carried out, and the manganese content of the crop taken as an index of the availability. Eight pots in duplicate of the Glen Osmond soil were treated with either hydrochloric acid or calcium carbonate in 1928 to give a range of soil reaction from about pH 5·5 to pH 8·4. These were sown with Algerian oats in 1929, twelve months after treatment of the soil, and the crop harvested when at the flowering stage. The crop from each pair of pots was analysed and its manganese content determined. The soil reaction at the time of seeding was also determined. The results are shown diagrammatically in Fig. 1.

It will be seen that there was a steady decrease in the total amount of manganese (expressed in mg.) absorbed by the crop of each pot as the soil reaction varied from pH 5·7 to about pH 7·0. In the pots more alkaline than this the amount of manganese in the crop decreased only slightly. In the dry weight of the crop itself the decrease was from 129 p.p.m. at pH 5·7 to 31·3 p.p.m. at pH 8·3.

Some results, secured in 1928, on the amount of manganese absorbed by oats growing on the Mt Gambier soil, the pH of which had been adjusted by the use of increasing amounts of hydrochloric acid, are also included in Fig. 1. In this case 4·4 mg. of manganese were removed per pot by the crop growing in the most acid soil (pH 5·6), and this amount rapidly fell to 0·11 mg. at pH 7·0 and 0·03 mg. in the next two sets of pots. The oats in these latter pots showed severe symptoms of manganese deficiency.
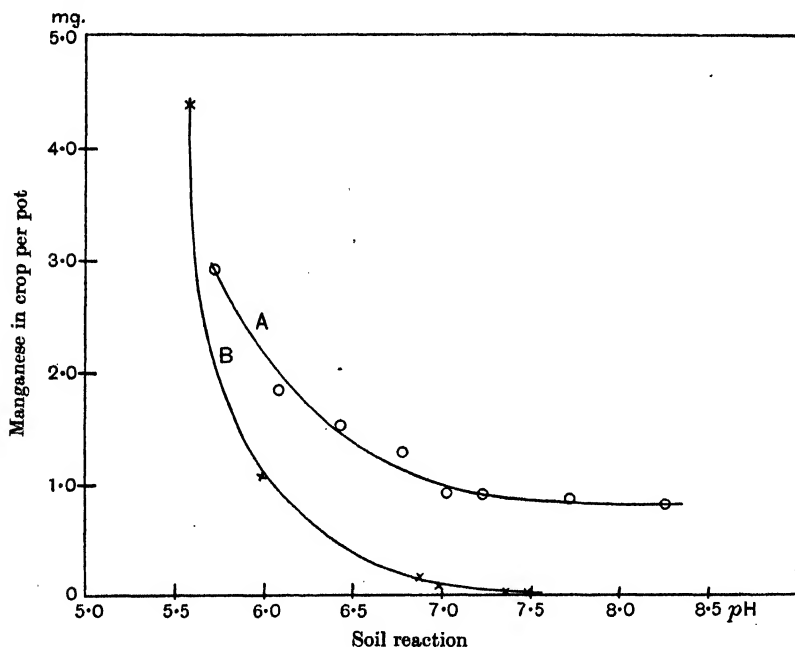

Fig. 1. Showing the relation between soil reaction and the amount of manganese absorbed by oats growing on two soils. A, Glen Osmond soil series. B, Mt Gambier soil series. The plants in those pots of Mt Gambier soil of pH 7·4 to 7·5 exhibited typical symptoms of manganese deficiency.

From both series of pots it will be seen that the availability of manganese is largely increased by increasing the acidity of the soil. The difference between the Glen Osmond and the Mt Gambier soils in the pots near neutrality is remarkable in that it shows that two soils of the same reaction and approximately the same amount of total manganese should have such different amounts of available manganese. The next series of pot experiments was designed to gain more information on factors controlling the availability of manganese in neutral or alkaline

soils. Since the solubility due to the reaction of the soil alone will be low, the availability must be influenced by some other factor in association with the soil reaction.

The four soils previously described were filled into a series of glazed earthenware pots each holding about 10 kg. The pots were filled, at the end of April, with moist soil which was tamped to secured even packing. The water-holding capacity of each soil was determined by the method of Keen and Raczkowski (1921), and, taking this value to represent 100 per cent. saturation, sufficient water was added to each pot to bring it to a predetermined degree of saturation. The pots were seeded with Algerian oats on June 2, 1930, and, after germination, thinned to six seedlings each. Each pot was then covered with a $1\frac{1}{2}$ inches layer of gravel to minimise evaporation from the soil surface. All pots were weighed weekly and water added to replace losses. As growth advanced these transpiration losses were replaced twice and later thrice weekly. All pots received a uniform dressing of 0·5 gm. of pure monocalcic phosphate and 1·5 gm. of ammonium nitrate each. The crop was harvested at the flowering stage, or, in the case of badly diseased and stunted plants, on the same date as the corresponding healthy plants. Manganese was determined in all the crop samples after harvesting. The pots were kept in the open except during rain and at night, when they were in the glasshouse.

In the first series duplicate pots of each soil type were maintained at 45, 60, 75 and 90 per cent. of their water-holding capacity. In a second series the three manganese-deficient soils were treated in the following ways, the treatments being carried out in duplicate and at both 60 and 90 per cent. water saturation[1].

(a) manganese sulphate added at the rate of 1 cwt. per acre;

(b)   ,,   ,,   ,,   5 cwt.   ,,

(c) soil waterlogged for a week, then allowed to dry out to 60 or 90 per cent. saturation before seeding and then maintained at these values.

The results of some of the pots are illustrated in Figs. 2 and 3, and Table III gives the average yield per pot and also the average amount of manganese in the crop, expressed first as parts of manganese per

[1] Additional pots were made up to test the possible effects of copper sulphate, ferrous sulphate and sodium chloride. As the results obtained proved to have no immediate bearing on the problem of manganese availability, they are not discussed here (see also Figs. 2 and 3).

Fig. 2. Pot experiments on the effect of the degree of soil saturation on the growth of oats in the volcanic ash soil from Mt Gambier, South Australia. A 2, 45 per cent. water saturation; A 4, 60 per cent. water saturation; A 6, 75 per cent. water saturation; A 7, 90 per cent. water saturation. In the first three pots the oats failed through manganese deficiency.

Fig. 3. Pot experiments on the growth of oats in the calcareous soil from Corney Point, South Australia. All pots were maintained at 60 per cent. saturation and the following soil treatments were carried out: A 24, untreated; B 54, manganese sulphate added at the rate of 1 cwt. per acre; B 56, manganese sulphate added at the rate of 5 cwt. per acre; B 58, sodium chloride added equivalent to 0·1 per cent. of the soil; B 60, copper sulphate added at the rate of 2 cwt. per acre; B 62, ferrous sulphate added at the rate of 1¼ cwt. per acre; B 64, soil waterlogged for 1 week and then allowed to dry to 60 per cent. saturation before seeding.

Table III. *Showing the dry weight and manganese content of Algerian oats grown on different soils in the 1930 pot experiment.*

Soil treatment	Mt Gambier soil			Penola soil			Corney Point soil			Glen Osmond soil		
	Av. yield per pot gm.	Mn in dry matter p.p.m.	Mn in dry matter mg. per pot	Av. yield per pot gm.	Mn in dry matter p.p.m.	Mn in dry matter mg. per pot	Av. yield per pot gm.	Mn in dry matter p.p.m.	Mn in dry matter mg. per pot	Av. yield per pot gm.	Mn in dry matter p.p.m.	Mn in dry matter mg. per pot
45 % water saturation	1·1*	6·4	0·007	11·6*	8·5	0·098	6·0*	4·8	0·029	50·0	43·4	2·17
60 % water saturation	1·5*	5·3	0·008	15·2*	9·9	0·151	6·3*	7·6	0·048	81·0	37·0	3·00
(a) 1 cwt. MnSO₄ per acre	87·2	17·3	1·51	54·0	11·5	0·62	66·0	11·1	0·73	—	—	—
(b) 5 cwt. MnSO₄ per acre	77·0	46·0	3·54	68·3	48·2	3·29	76·5	23·5	1·80	—	—	—
(c) waterlogged† before seeding	75·0	42·3	3·17	23·4‡	7·0	0·16	55·8	13·1	0·73	—	—	—
75 % water saturation	3·2*	6·3	0·020	49·0‡	6·4	0·311	12·8*	7·0	0·090	112·7	36·6	4·13
90 % water saturation	37·6	14·4	0·54	58·5‡	7·7	0·450	59·8	45·5	2·72	130·0	39·1	5·08
(a) 1 cwt. MnSO₄ per acre	82·1	26·3	2·16	79·2	10·2	0·81	82·1	26·7	2·19	—	—	—
(b) 5 cwt. MnSO₄ per acre	79·8	55·3	4·41	89·5	30·8	2·76	79·2	43·7	3·46	—	—	—
(c) waterlogged† before seeding	98·9	56·7	5·61	70·9	8·9	0·63	48·4	69·4	3·36	—	—	—

* Oats showed severe symptoms of manganese-deficiency disease at time of harvesting.

† These pots were waterlogged for one week and allowed to dry to 60 and 90 per cent. saturation respectively.

‡ Oats showed some symptoms of manganese-deficiency disease at time of harvesting.

million parts of dry matter and secondly as milligrams of manganese in the crop of each pot.

As will be seen from the table the crop yield on the manganese-deficient soils was enormously increased either by high water saturation of the soil, by dressings of manganese sulphate, or by waterlogging the soil prior to seeding. The increased growth was accompanied by freedom from the symptoms of manganese deficiency which were so very pronounced in the pots of low water saturation. Some symptoms of manganese deficiency did appear in the untreated pots at 90 per cent. water saturation, at an early stage of growth, but the plants later recovered and made quite healthy growth. No symptoms whatever appeared in any of the pots treated with manganese sulphate nor in those pots of the Mt Gambier and Corney Point soils which were waterlogged prior to seeding. In the pots kept at 60 per cent. saturation the increased yield due to applications of manganese sulphate averaged 830 per cent., and that due to the preliminary waterlogging was 570 per cent.

The manganese content of the crop of each pot shows some interesting relationships. Although the amount of manganese in parts per million fluctuated in the pots in the degree of saturation series, it was always

lower in those cases where disease occurred (5·3–9·9 p.p.m.). It was significantly higher in the 90 per cent. saturation pots of the Mt Gambier soil (14·4 p.p.m., little disease only showing at harvesting) and Corney Point soil (45·5 p.p.m., plants quite healthy). Likewise all plants grown in the Glen Osmond soil were high in manganese. If the amount of manganese (in mg.) in the crop is examined, a consistent increase is observed as the degree of saturation was increased from 45 to 90 per cent. for each soil. The increase is again most marked in the case of the Mt Gambier and Corney Point soils at 90 per cent. saturation. Since the supply of available manganese is the factor limiting growth in these experiments[1], the amount of manganese absorbed by the plants may be taken as some measure of the amount of available manganese in the soil.

Water-culture experiments (unpublished) have shown that if manganese be available to the plant in solution, high percentage recoveries (60–80 per cent.) may be expected, the plant absorbing manganese in quantities much greater than appear to be necessary for normal growth. Thus the greater quantities of manganese recovered (together with the healthy growth of plants) at 90 per cent. water saturation of the soil are held as evidence of the greater availability of manganese at this saturation. Likewise the amount of manganese removed by the crops from the soils waterlogged and then maintained at 90 per cent. saturation is greater than the controls at 90 per cent. saturation. The amount absorbed by the crops in the pots waterlogged and then kept at 60 per cent. saturation is somewhat less. These facts can all be explained on the assumption that manganese as manganese dioxide is unavailable to plants until it is reduced to a lower state of oxidation. Soils at 90 per cent. water saturation will be subject to conditions more favourable to biological reduction than those at 45 per cent. saturation owing to the smaller amount of air in the pore space of the former. These conditions will favour the reduction of manganese dioxide to manganous compounds. Likewise, waterlogging the soil is very favourable to the reduc-

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[1] Further proof of the fact that manganese alone is the element limiting growth was obtained from a series of untreated pots maintained at 60 per cent. saturation. After the oats had been showing severe symptoms of disease for some weeks manganese sulphate solution was brushed on to the leaves of the plants in half the pots, taking precautions to avoid letting any manganese sulphate reach the soil. The other pots were left untreated as controls. After a few days those plants treated with the manganese sulphate solution started to make fresh healthy growth and within a fortnight were quite vigorous, whereas the untreated plants had not changed at all. Thus the plants were able to secure all the other mineral elements from the "affected" soils except manganese and when this was supplied through the leaves growth became normal.

tion of manganese dioxide to manganous compounds as was shown in the laboratory investigations, and if the soil is then kept at 90 per cent. saturation more of the reduced manganese will remain as such than in the pots at 60 per cent. saturation, for the oxidising power of the soil will be greater at this lower saturation. That soluble manganese salts are converted to less available compounds on addition to the soil (as was shown previously) is confirmed by the very low recovery of manganese by the plants in the pots to which manganese sulphate was added. The highest recovery from the addition of 0·54 gm. of $MnSO_4 4H_2O$ per pot (equivalent to 1 cwt. per acre) was only 2·19 mg. manganese, and from five times this quantity only 4·41 mg. was absorbed by the plants. Table III shows that applications of manganese sulphate have always increased both the relative and the total amounts of manganese in the dry matter compared to the control pots.

Thus soil conditions that favour reduction processes (high degree of water saturation) have been shown to increase the amount of manganese available to the crop, and conversely conditions favouring oxidation (low water saturation) tend to lessen the amount of manganese available.

In an earlier publication (Samuel and Piper, 1929) it was thought that tight packing of the soil may have influenced the availability of manganese. A further experiment was carried out during 1930 in which two pots were filled with moist Mt Gambier soil, loosely packed into the pots and two pots were filled with air-dry soil tamped in tightly. Water was added to both sets of pots to bring them to 60 per cent. saturation, and they were maintained at this moisture content throughout the experiment. There was slight swelling on moistening the air-dry soil, thus increasing the degree of packing. The pots were seeded at the same time as those of the other series. Disease appeared at an early stage, becoming equally severe in all four pots, and the plants made very little growth. Thus the degree of soil packing of itself does not affect the availability of manganese, and it appears that the earlier results were probably due to impeded drainage following the tight packing and leading to temporary reducing conditions within the soil. This could not happen in the case of the soils maintained at 60 per cent. water saturation, but the moisture content of the soils of the earlier experiments was not controlled.

III. GENERAL DISCUSSION.

Part of the manganese of a soil occurs in certain ferro-magnesian minerals and other complex silicates. Some of these are so insoluble that they are not readily decomposed, even by concentrated hydrochloric

acid, and therefore they need not be considered in this discussion of the forms of available manganese. Neither manganese silicate nor manganese carbonate are likely to be present in highly weathered soils; Robinson (1929) could not detect either of these in the silt and sand fractions of two soils examined by him. The two forms in the soil which are important as the source of manganese for plants are replaceable manganese (*i.e.* bivalent manganese ions in the absorption complex) and manganese dioxide. The experimental work described herein has brought out the fact that the relative amount of these two forms is controlled by the soil reaction and the oxidation-reduction equilibrium, in intimate association, and that only when these factors are favourable to the presence of bivalent manganese is it available to plants. The availability of manganese in those soils which do not normally carry healthy growth and in which plants are subject to manganese-deficiency diseases, can be increased greatly, either by increasing the soil acidity or by promoting reducing conditions in the soil, such as those brought about by temporary waterlogging. The increased availability following both treatments is reflected in the healthy, increased growth of the plants and in the much greater amount of manganese absorbed by them as compared with those growing on untreated soils.

That soil reaction is a factor in determining the availability of manganese has been recognised for many years, but it is only quite recently that much work has appeared on the influence of the oxidation-reduction equilibrium. Even then, most of this latter work has dealt with the development of toxic concentrations of soluble manganese in the soil solution. The opportunity of studying several soils on which plants are subject to a manganese-deficiency disease has now shown that the oxidation-reduction equilibrium is also important in determining the availability of the small amounts of manganese necessary for plant growth.

The precipitation of manganese from solutions of manganous salts has been carefully investigated by Britton (1925, 1927 *a, b*) under conditions ensuring the absence of the more highly oxidised compounds and he has shown that the first precipitation of manganous hydroxide occurs at pH 8·41, of manganous silicate at pH 7·35 and of manganous phosphate at pH 5·76. Carr and Brewer (1923) had found that the hydroxide began to precipitate at pH 7·2. They do not indicate whether any precautions were taken to exclude air, and it seems probable that their precipitate, appearing at a lower pH than that of Britton's, may have been manganic hydroxide ($Mn(OH)_3$). This latter is precipitated when

an ammoniacal solution of a manganous salt is exposed to the air. It is therefore seen that in soil solutions containing silicate and phosphate ions, and under aerobic conditions, the solubility of manganese will be decreased as the soil reaction is changed from acid to alkaline conditions.

Mann (1930) studied the effect of additions of calcium and magnesium carbonates on the water solubility of manganese in two acid soils. He found that the solubility was rapidly decreased by both carbonates as the pH of the soil increased, only a trace of water-soluble manganese being detected at pH 7·0. He also determined the amount of manganese in crops growing on one of these soil types, treated with increasing quantities of calcium and magnesium carbonates. The increasing dressings of the carbonates steadily decreased the amount of manganese in the crop until near neutrality the plants exhibited typical symptoms of manganese deficiency. Godden and Grimmett (1928) also found that applications of calcium carbonate decreased the amount of manganese absorbed by plants growing on the soils. The lime-induced chlorosis of Gilbert, McLean and Hardin (1926) was accompanied by a low manganese content of plants growing on the limed soils, as compared with those growing on more acid soils. The lower solubility of manganese in alkaline soils was also found by McHargue (1923), who showed by means of pot experiments that amounts of added manganese sulphate that were toxic in acid soils were rendered less toxic or even beneficial on similar soils neutralised with lime. Schollenberger (1928) found that calcite crystals mixed with an acid soil caused a precipitation of manganese, the calcite crystals becoming covered with a deposit of manganese dioxide. Deatrick (1919) also found that, on addition to a soil, soluble manganese compounds were converted to the dioxide according to the basicity of the soil.

Truog (1916) and Funchess (1918) have observed that applications of lime to acid soils decreased the amount of replaceable manganese. Changes in replaceable manganese at different soil reactions have also been observed by Ruprecht and Morse (1917) and Schollenberger and Dreibelbis (1930). They noted increases in replaceable manganese in field plots following the increased acidity due to the continued use of ammonium sulphate fertiliser. Like Truog and Funchess they also found large decreases in replaceable manganese on liming the soils to neutrality.

Connor (1918) found significant increases in the amount of replaceable manganese on flooding a soil, and Schollenberger (1928) and Metzger (1930) reported similar increases. The latter found that replaceable manganese was significantly higher in a twenty-year-old rice field than

in an adjoining non-irrigated soil. He also showed that storing a soil at 20 per cent. moisture content (*i.e* under conditions favouring oxidation) caused a decrease in the replaceable manganese. The increase in soluble manganese brought about by waterlogging has also been noted by Godden and Grimmett (1928), who showed that plants grown in undrained pots were very much richer in manganese than those in drained pots. Robinson (1929) found a large increase in water-soluble manganese on waterlogging a soil, and stated that soluble manganese is dependent on soil reaction and whether oxidising or reducing conditions prevail. More recently (1930) he has shown that submerged soils were characterised by strongly reducing conditions. Hydrogen was found in the gases produced and the soil solutions contained large amounts of soluble iron and manganese compounds. Sulphides were also present. The reducing conditions developed very rapidly on submerging a normal soil. Brewer and Carr (1927) have attributed some of the beneficial effects of farmyard manure on certain soils to the reduction of manganese dioxide to manganous compounds, and Schollenberger and Dreibelbis (1930) have shown that exchangeable manganese has been significantly increased in field plots by applications of stable manure extending over 32 years. Schollenberger (1930) has also found that toxicity caused by leaking natural gas in a soil was due to an excessive amount of replaceable manganese. The anaerobic conditions brought about by the leaking gas caused the reduction of manganese dioxide to the manganous form, which was more active in the soil. Storing the moist soil under aerobic conditions caused the reduced manganese to revert to the oxidised form.

The influence of the oxidation-reduction equilibrium on the availability of manganese now offers a probable explanation of a number of observations, made at different times, in connection with the occurrence of manganese deficiency. In earlier work (Samuel and Piper, 1928) it was shown that partial sterilisation, by heat, of soils deficient in available manganese enabled healthy crops to be grown, and very considerably increased the amount of soluble manganese in the water percolates of such soils. The much greater biological activity, following the partial sterilisation, probably affects the oxidation-reduction equilibrium, thus increasing the proportion of manganous ions present in the soil solution. In the field manganese-deficiency disease has been observed to be worse in dry years than in wet years. It seems probable that in wet years conditions of temporary waterlogging of the soil may prevail for a sufficient period to affect the oxidation-reduction equilibrium and so increase the availability of manganese. Similarly, low-lying patches,

which frequently become wetter than the rest of the field, have been noticed to be less subject to the disease.

An increase in the severity of manganese deficiency, following the use of nitrate fertilisers, has been found by Arrhenius (1924). He also found that applications of manganese dioxide to the soil made the disease worse. Both of these substances, being oxidising in nature, tend to decrease the availability of manganese. Arrhenius' observation that heavy dressings of stable manure were beneficial is again explained by the accompanying increased activity in the microbiological life of the soil, tending to move the oxidation-reduction equilibrium in the direction of greater reduction. In this connection the possibility of increasing the availability of manganese by green manuring needs to be investigated. Although certain crops (oats and barley in particular) may fail entirely, other plants (rye, grasses, legumes, etc.) may make sufficient growth to be used as a green manure crop, and this may lead to an economic method of field control of manganese deficiency.

The writer desires to acknowledge his indebtedness to Prof. J. A. Prescott for the many helpful suggestions throughout the course of this investigation.

IV. SUMMARY.

Recent work on the solubility of manganese compounds in the soil, leading to a general consensus of opinion that the solubility of this element is intimately controlled by the soil reaction and by the oxidation-reduction equilibrium, is shown to have an important bearing on the availability of this element to plants. This has been established by means of laboratory tests and pot experiments on three different soil types, characteristically deficient in available manganese. It has been shown that normal plant growth can be established on such soils either by increasing the acidity of the soil or by subjecting the soil to reducing conditions such as those brought about by high water saturation or temporary waterlogging. On these soils the increases in growth amounted to several hundred per cent. These responses are in every respect similar to those obtained by the application of manganese sulphate to the soils.

In this series manganese deficiency failure is overcome most successfully by the application of manganese sulphate or by waterlogging prior to seeding.

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FURTHER OBSERVATIONS ON THE NITROGENOUS MANURING OF GRASSLAND.

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IN a previous paper published in this *Journal*(1) an account was given of some investigations on the nitrogenous manuring of old-established permanent grass under a system of rotational grazing which was begun in 1927 and continued in 1928. The chief points discussed in that paper were (1) the methods adopted to ensure a reliable sampling of the herbage, (2) the evidence of a definite suppression of leguminous species by sulphate of ammonia, (3) the relative crude protein and moisture contents of grasses, clovers and "other species." During the subsequent two years the investigations were continued on practically the same lines as in 1928, and in the present paper the results of the three seasons 1928, 1929, 1930 are incorporated.

For a description of the soil, management and arrangement of the plots reference should be made to the earlier paper. It will suffice to state here that, out of nine five-acre plots managed on a rotational system, four were selected for detailed observations; two of these received periodical dressings of sulphate of ammonia and two received none; otherwise the treatment of all four plots was the same. In 1928 there were four grazing rounds, in 1929 and 1930 three (apart from a very light preliminary grazing with sheep in March), and in each year there were three applications of sulphate of ammonia, each one cwt. per acre, the times of application being approximately February, May and July. The first complete grazing commenced in 1928, 1929, 1930, on the dates April 26, April 27, April 28 respectively, and in each year the final grazing was in September or October.

Basal manuring. Every year half of each plot was dressed with a mixture of mineral phosphate and potash salts, so that in the four years since the experiment started the whole area had been treated twice. Independent tests showed that there was an ample supply of these two "minerals." Lime was applied to one-fifth of each plot annually, although *p*H determinations showed that the soil was neutral to slightly

alkaline in reaction at the outset and was well supplied with exchange-able calcium. It was considered, therefore, that lime, phosphoric acid and potash could not have acted as limiting factors.

Technique. For the economic data a daily record of all animals grazing the plots was kept, and from this by means of a table of equivalents the total number of cow-days of grazing provided by each plot was obtained. As pointed out previously, it was considered that such figures were subject to considerable errors and for a single year could be but a rough guide; over a period of years, however, they may provide useful information.

The method of sampling the herbage was fully described in the earlier paper with the aid of a diagram. As it was considered impossible with the time and labour available to sample adequately the whole of the four five-acre plots, attention was concentrated on selected half-acre areas in each plot, the principal aim being to trace any changes which might result from the management and manuring. The half-acre areas were subdivided into four strips and, from these, nine random one-square-yard samples (thirty-six in all) were cut on each occasion when the plots were ready for grazing. The samples were taken to the laboratory in waterproof bags and, after weighing, each set of nine was used to make up a strip-composite; from these four composite samples portions were taken for chemical and botanical analysis.

Sampling error. It was shown in the previous paper that if thirty-six square-yard samples were taken, the "probable error" of the mean was not above 4 per cent. of the mean, except when the herbage was so short that there was very little to be sampled.

In 1930 on the suggestion of Dr Wishart of Rothamsted a better test of the sampling error was made by taking two independent sets of sixteen samples (four from each strip) on each occasion of sampling. Since there were three grazing rounds in that year and four plots, this method gave twelve pairs of means (each calculated from sixteen samples) from which to calculate the error. It was found that the standard error of the mean of the pairs of determinations was 4·75 per cent., a satisfactorily low figure. Since in 1928 and 1929 thirty-six square-yard samples were taken, the error in those years would be slightly lower.

Weather. The chief weather factor bearing on yields of grass is rain-fall, and probably the next most important is the temperature during the early months of the year. Since the October, November and December rainfall cannot contribute to the grazing of the same calendar year the figures given in Table I (a) are for "harvest" years, October to

September inclusive. The year has been divided into three sections: winter (October to March), spring (April to May), and summer (June to September), since this subdivision serves to bring out very clearly the chief differences between the three seasons concerned. To illustrate temperature differences the readings, "One foot in ground" are given, for the first five months of the calendar year, in Table I (b).

Table I (a). *Rainfall.*

Harvest year	Winter rain (Oct.–March) (in.)	Spring rain (April–May) (in.)	Summer rain (June–Sept.) (in.)	Total (in.)
1928	15·8	2·1	6·65	24·55
1929	11·05	3·2	3·5	17·75
1930	20·15	4·5	9·3	33·95

Av. for 3 years, 25·4 in.

Table I (b). *Soil temperature in early months.*

Year	Lowest reading 1 foot in ground (°F.)					Monthly average reading 1 foot in ground (°F.)				
	Jan.	Feb.	March	April	May	Jan.	Feb.	March	April	May
1928	36	37	39	44	51	39	40	43	47	53
1929	35	33	34	42	45	36	35·5	38	44	53
1930	39	37	39	44	51	41	38	41	47	54

It will be seen from these figures that although the total rainfall for the 1928 season was not much below normal (the average annual rainfall at "Oaklands" is about 26 in.), nearly two-thirds of this fell in the winter period, while the two "spring" months were exceptionally dry. The early calendar months were comparatively mild, the temperature 1 foot in the ground averaging 43° F. in March with a minimum reading 39°.

In the 1929 season the rainfall was about two-thirds of the average and of this less than 7 in. fell during the six months April to September: that is, the season was extremely dry. As regards temperature, this year was also remarkable, as the winter was the most severe since 1895. This is well shown by the soil temperature readings: even in April the average reading was still 3° below that of the corresponding month in 1929 and 1930.

In most respects the 1930 season was a direct contrast to 1929. The rainfall was approximately double; the winter fall reached the high figure of 20·15 in. and the summer amount was nearly 14 in. The winter was mild, but owing to the sodden condition of the ground the soil was a little slow in warming up. Throughout the season there was a plentiful supply of moisture.

From the records made in the diary the total grazing is expressed in terms of cow-days by means of the following scale of equivalents:

> 1 cow = 1 horse.
> = 1 two-year old cattle.
> = 2 yearling cattle.
> = 7 half-bred sheep.
> = 10 cross-bred lambs.

The cow-days of grazing for the four plots concerned are shown in Table II.

Table II. *Grazing in cow-days per acre.*

		1928	1929	1930	Average 3 years
Nitrogen	2	197	161	241	200
Plots	7	189	155	190	178
	Average ...	193	158	215·5	189
Control	3	150	119	199	156
Plots	8	168	113	171	151
	Average ...	159	116	185	153·5
Average of all...	...	176	137	200	171
Rainfall (in.) ...	...	24·55	17·75	33·95	25·4

The agreement between the no-nitrogen plots is fairly close in each year and also that between the nitrogen plots except in 1930. For the effect of the nitrogen, Plot 2 is to be compared with its neighbour 3, and 7 with 8. On the average for the three years, Plot 2 has yielded 28 per cent. more grazing than Plot 3 and Plot 7 18 per cent. more than Plot 8. Putting the two nitrogen plots together they have given 23 per cent. more grazing than the two controls with a variation from 16·5 in 1930 to 36 per cent. in 1929. The absolute, as well as the percentage, increase was greatest in the driest year.

The influence of rainfall is shown at the bottom of the table by averaging the cow-days for all the plots in each year. In 1930 the production is 46 per cent. above the dry season 1929: on the nitrogen plots the difference is 36·5, on the controls 60 per cent., the actual differences being 57·5 and 69 cow-days respectively.

THE WEIGHT OF HERBAGE PRODUCED AND ITS BOTANICAL COMPOSITION.

Procedure. At least two portions of the composite sample of herbage obtained by the methods already described were divided into the four groups (1) grass species, (2) leguminous species, (3) other species, (4) dry

Table III. *Yield of herbage and its botanical composition. All figures are cwt. per acre of dry matter.*

		Plot 2 (nitrogen)							Plot 3 (no nitrogen)						
Year Round		Date sampled	Gross total	Grasses	Clovers	Weeds	Waste	Net total	Date sampled	Gross total	Grasses	Clovers	Weeds	Waste	Net total
1928	1	30.iv	7·82	6·50	0·06	0·48	0·78	7·04	7.v	8·80	6·68	0·27	1·08	0·77	8·03
	2	25.vi	15·27	12·78	0·44	0·53	1·54	13·73	29.vi	14·82	11·87	1·20	0·82	0·93	13·89
	3	2.viii	2·67	1·17	0·03	0·02	1·45	1·22	6.viii	4·63	1·77	0·22	0·32	2·32	2·31
	4	20.ix	4·12	2·77	0·02	0·04	1·29	2·83	24.ix	3·80	2·02	0·09	0·17	1·52	2·28
Year's total		—	29·88	23·20	0·55	1·07	5·06	24·82	—	32·05	22·34	1·78	2·39	5·54	26·51
1929	1	30.iv	7·80	5·65	0·06	0·42	1·67	6·13	3.v	8·50	4·30	0·28	0·35	3·57	4·93
	2	6.vi	20·00	18·42	0·28	0·84	0·46	19·54	11.vi	15·30	11·52	1·59	1·76	0·43	14·87
	3	16.ix	6·20	2·03	0·04	0·02	4·11	2·09	18.ix	5·50	1·23	0·08	0·69	3·50	2·00
Year's total		—	34·00	26·10	0·38	1·28	6·24	27·76	—	29·30	17·05	1·95	2·80	7·50	21·80
1930	1	29.iv	14·55	8·37	0·03	0·68	5·47	9·08	5.v	12·60	8·00	0·20	1·25	3·15	9·45
	2	11.vi	24·18	22·56	0·06	1·00	0·56	23·62	19.vi	23·58	20·44	0·97	1·54	0·63	22·95
	3	29.viii	19·48	16·76	0·23	0·15	2·34	17·14	8.ix	15·86	12·02	0·97	0·62	2·25	13·61
Year's total		—	58·21	47·69	0·32	1·83	8·37	49·84	—	52·04	40·46	2·14	3·41	6·03	46·01
Annual average		—	40·70	32·33	0·42	1·39	6·56	34·14	—	37·80	26·61	1·96	2·87	6·36	31·44

		Plot 7 (nitrogen)							Plot 8 (no nitrogen)						
Year Round		Date sampled	Gross total	Grasses	Clovers	Weeds	Waste	Net total	Date sampled	Gross total	Grasses	Clovers	Weeds	Waste	Net total
1928	1	14.v	7·85	6·18	0·07	0·59	1·01	6·84	18.v	7·62	5·93	0·29	0·83	0·57	7·05
	2	6.vii	16·34	14·03	0·19	0·55	1·57	14·77	11.vii	7·05	5·84	0·40	0·21	0·60	6·45
	3	9.viii	7·64	4·41	0·06	0·16	3·01	4·63	14.viii	6·05	3·60	0·25	0·12	2·08	3·97
	4	27.ix	4·20	1·56	0·03	0·05	2·56	1·64	2.x	2·33	0·86	0·06	0·04	1·37	0·96
Year's total		—	36·03	26·18	0·35	1·35	8·15	27·88	—	23·05	16·23	1·00	1·20	4·62	18·43
1929	1	7.v	11·05	5·72	0·06	0·75	4·52	6·53	11.v	7·75	4·55	0·46	1·05	1·69	6·06
	2	20.vi	23·40	21·32	0·29	1·11	0·68	22·72	28.vi	16·90	12·44	1·74	1·30	1·42	15·48
	3	23.ix	8·30	1·09	0·02	0·07	7·12	1·18	26.ix	5·20	0·77	0·10	0·05	4·28	0·92
Year's total		—	42·75	28·13	0·37	1·93	12·32	30·43	—	29·85	17·76	2·30	2·40	7·39	22·46
1930	1	9.v	15·75	13·00	0·09	1·12	1·54	14·21	15.v	15·42	8·51	0·99	1·55	4·37	11·05
	2	5.vii	22·14	18·97	0·29	0·80	2·08	20·06	11.vii	17·02	11·22	1·41	0·97	3·42	13·60
	3	12.ix	19·41	15·98	0·61	0·59	2·23	17·18	21.ix	18·84	12·62	2·75	0·55	2·92	15·92
Year's total		—	57·30	47·95	0·99	2·51	5·85	51·45	—	51·28	32·35	5·15	3·07	10·71	40·57
Annual average		—	45·36	34·09	0·57	1·93	8·77	36·59	—	34·73	22·11	2·82	2·22	7·58	27·15
Ann. average two nitrogen plots			43·03	33·21	0·50	1·66	7·66	35·37	Am. av. two control plots	36·26	24·36	2·39	2·54	6·97	29·29

material, dung, earth, etc.: for brevity they have been designated "Grasses," "Clovers," "Weeds" and "Waste." These were weighed while still fresh, then dried at 100° C. and weighed again. It was possible, therefore, to calculate the weight per acre of each group in the herbage at every sampling in the three years concerned. The results are set out in Table III.

As the amount of "waste" is largely dependent on the weather conditions at the time of sampling, the figures for this component have been subtracted from the gross weights of dry herbage to give the columns headed "net total." These net total figures are to be regarded as the best estimate obtained of the edible dry matter produced; they are summarised in Table IV.

Table IV. *Yearly totals and averages of net dry matter. Cwts. per acre.*

	1928	1929	1930	Annual average
Plot 2 (N)	24·82	27·76	49·84	34·14
Plot 3 (no N)	26·51	21·80	46·01	31·44
Plot 7 (N)	27·88	30·43	51·45	36·59
Plot 8 (no N)	18·43	22·46	40·57	27·15
Year's av. all plots... ...	24·41	25·61	46·97	32·33
Year's rainfall (in.) ...	24·55	17·75	33·95	

$$\text{Ratio} \frac{\text{Plots } 2+7}{\text{Plots } 3+8} = \frac{70\cdot73}{58\cdot59} = \frac{121}{100}.$$

Effect of rainfall on yield. The general correspondence between yield and rainfall is brought out by putting alongside one another the rainfall figures from Table I and the average yield of net dry matter per grazing round based on Table III. This is done in Table V.

Table V. *Correspondence between rainfall and yield.*

	1928	1929	1930
Winter rainfall (Oct.–March) (in.)	15·8	11·05	20·15
Average net total dry matter 1st grazing round (cwt.)...	7·24	5·19	10·95
Spring rainfall (April–May) (in.)	2·1	3·2	4·5
Average net total dry matter 2nd grazing round (cwt.)...	12·21	18·15	20·06
Summer rainfall (June to mid-Sept.) (in.)	6·35	3·25	6·8
Average net total dry matter 3rd grazing round (cwt.)...	4·96*	1·55	15·96

* 3rd + 4th grazing rounds in this year.

The rather large difference between the 2nd grazing round yields in 1928 and 1929 (nearly 6 cwt.) is possibly to be attributed to the "lateness" of the 1929 season, although it may also be connected with the distribution of the rain. In May, 1928, there were 11 days on which rain was recorded but no fall was over 0·25 in., whereas in May, 1929,

there were 13 wet days including 4 over 0·25 in. Similarly for the 3rd grazing round the very big difference between 4·96 cwt. for 1928 and 15·96 cwt. in 1930 is possibly to be accounted for not only by the greater reserves of soil moisture in 1930 but also by the much larger number of heavy falls in the latter year during the summer months as compared with 1928 (during the months July to September, 6 falls over 0·25 in. in 1928 but 15 in 1930). Very light falls of rain in the summer months evaporate without producing any appreciable effect, so that rainfall totals may be misleading as measures of effective rain.

Effect of nitrogen on yield. For the measurement of the effect of the application of 3 cwt. of sulphate of ammonia per annum per acre only the two pairs of plots, 2 and 3, 7 and 8, are available, so that, in spite of the accuracy of the sampling method used, the inadequacy of replication makes it impossible to attach a precise degree of significance to the results. For the three years, as shown in Table IV, the nitrogen plots average 21 per cent. more than the controls. For the individual years, 1928, 1929, 1930 this increase was 22, 31·5, and 17 per cent. respectively, the highest figure being in the driest year. In 1928 the nitrogen Plot 2 is actually 6·4 per cent. below 3, but in the same year 7 is 51·4 per cent. above 8. In spite of this lack of agreement between the pairs of plots, it is considered that the average increase for the three years is between 20 and 30 per cent. This is supported by the following further facts and considerations.

During the seasons 1929 and 1930 a small "Latin square" of ungrazed grass on Plot 9 was cut at three-weekly intervals. In the former year plots receiving periodical dressings of sulphate of ammonia (total for year 4·5 cwt. per acre) gave a significant increase of 76 per cent. over the no-nitrogen plots: in 1930 the difference was 54 per cent. Assuming that under grazing conditions 30 cwt. of dry matter per acre are consumed containing 2·75 per cent. nitrogen and that 70 per cent. of this is excreted by the animals on to the grass, the latter receives about 0·6 cwt. nitrogen per acre, mainly in an available form. It seems reasonable to suppose, therefore, that grass which, being under grazing conditions, is already receiving 0·6 cwt. nitrogen per acre would give much less than a 50 per cent. increase as the result of the application of a further 0·6 cwt. nitrogen in the form of 3 cwt. sulphate of ammonia. The "Oaklands" results suggest that over a period of years the percentage increase due to 3 cwt. of sulphate of ammonia per acre applied at intervals to old-established grazing land in Hertfordshire is not likely to exceed 30: it may be a little higher in dry, and less in wet, seasons.

Constituents of the herbage. It was emphasised in the earlier paper that there could be no doubt about the depressing effect of nitrogen on the leguminous species as shown by the results of the first two years; the succeeding years have fully confirmed this. The average annual production of "clovers" on the two nitrogen plots has been 0·445 cwt. per acre as compared with 2·39 on the two controls, the ratio being 1 to 5·4. For the pair of Plots 7 and 8 the ratio in the four years of the experiment has been:

	1927 (May)	1928	1929	1930
$\dfrac{\text{Clovers Plot 8}}{\text{Clovers Plot 7}}$	1·15	2·86	6·22	5·20

Hence from a position of approximate equality at the outset the production of clover on the nitrogen plot has declined to about one-fifth of the control, most of the decline having taken place during the first two years. The position now seems to be nearly stable.

Table VI. *Annual weighted percentages of chief grass species.*

	Plot 2 (nitrogen)			Plot 3 (control)		
Year	1928	1929	1930	1928	1929	1930
Species						
Lolium perenne	37·00	51·55	51·35	41·85	58·95	57·20
Poa pratensis and *trivialis*, chiefly *triv.*	15·30	4·45	5·95	14·20	4·90	4·90
Agrostis spp. ...	15·45	20·70	17·40	14·30	14·95	16·20
Holcus lanatus...	7·60	3·55	1·50	5·65	2·90	3·10
Festuca ovina ...	3·70	5·10	8·95	5·10	5·40	9·90
Alopecurus pratensis ...	4·55	6·60	5·65	3·25	0·55	1·10
Other grass species* ...	16·40	8·05	9·20	15·65	12·35	7·60
Total first five...	79·05	85·35	85·15	81·10	87·10	91·30

	Plot 7 (nitrogen)			Plot 8 (control)		
Year	1928	1929	1930	1928	1929	1930
Species						
Lolium perenne	18·85	40·60	36·15	35·50	37·65	39·00
Poa pratensis and *trivialis*, chiefly *triv.*	24·80	10·35	8·90	17·20	5·75	6·05
Agrostis spp. ...	21·10	22·50	23·50	17·60	29·60	24·45
Holcus lanatus...	13·00	5·85	7·25	9·25	4·10	5·45
Festuca ovina ...	5·50	8·40	14·35	3·80	6·25	12·00
Alopecurus pratensis ...	2·30	2·10	1·55	1·40	1·50	1·35
Other grass species* ...	14·45	10·20	8·30	15·25	15·15	11·70
Total first five...	83·25	87·70	90·15	83·35	83·35	86·95

* Other species of grass were *Anthoxanthum odoratum, Cynosurus cristatus, Avena flavescens, Dactylis glomerata, Phleum pratense, Avena pratensis, Poa compressa, Festuca pratensis.*

The species composition of the "grasses" by weight (Table VI). On each occasion of sampling in the years 1928, 1929 and 1930 (with two unimportant occasions in 1928 when the total weight of "grasses" per acre was under 2 cwt.) portions of the "grasses" separated out from

carefully made up composite samples of herbage were subdivided into species, dried, and weighed. This subdivision was either in duplicate or quadruplicate, and it is believed that a fairly accurate estimate has been obtained of the contributions *by weight*, in cwt. per acre, of the various grass species at each of the grazing rounds, ten in all, in the three years concerned. The amount of data so obtained is very considerable, and for the sake of brevity it has been condensed into Table VI which gives *weighted* percentages of the chief grass species for each year. Thus the figure 51·55 for Perennial Rye Grass on Plot 2 in 1929 means that in this year, of the total *weight* of "grasses" produced on this plot during the grazing season, 51·55 per cent. was estimated to consist of this species.

The chief deductions to be made from the results are:

1. Five species, Perennial Rye Grass, Meadow Grass (Rough-Stalked), Bent, Yorkshire Fog, and Sheep's Fescue account for 80 to 90 per cent. of the grasses on every plot. Meadow Foxtail comes next with a maximum of 6 per cent. on any plot. The remaining 8 to 15 per cent. is distributed between about eight species.

2. Except on Plot 8, Rye Grass shows a considerable increase during the three years. Bent Grass remains stationary or increases slightly. Bent and Rye Grass together account for at least 60 per cent. of the total on all plots.

3. Meadow Grass shows a big decrease on every plot, the figure in 1930 being approximately one-third of that in 1928.

4. Sheep's Fescue has increased on all plots and apparently is still increasing.

5. Yorkshire Fog has declined considerably on all plots.

6. There is no evidence of differential effect due to nitrogen.

These results show very clearly that under the system of rotational grazing *Lolium perenne*, *Agrostis* spp., and *Festuca ovina* can hold their own while *Poa trivialis* and *Holcus lanatus* decrease rapidly. At "Oaklands" other species individually are of little importance, and in no case collectively account for more than 15 per cent. of the total weight of grasses.

"*Weed*" *species.* The chief species other than Gramineae and Leguminosae have been thistles (chiefly *Carduus arvense*), tall sorrel (*Rumex acetosa*), pig nut (*Conopodium denudatum*), milfoil (*Achillea millefolium*), woodrush (*Luzula campestris*), ribgrass (*Plantago lanceolata*), bulbous buttercup (*Ranunculus bulbosus*) and self-heal (*Prunella vulgaris*).

It is a point of some practical importance whether the rotational system of grazing tends to encourage the growth of "weeds" at the

expense of the grasses and clovers. Owing to the influence of season on yield it is not possible to be guided by the actual weight of weeds produced, but some evidence can be obtained from the ratio of these to one of the other constituents, *e.g.* grasses. This ratio, calculated from Table III, is given in Table VII.

Table VII. *Ratio* $\dfrac{weight\ of\ "weeds"}{weight\ of\ "grasses"}$.

	Year... Plot	1927	1928	1929	1930
Nitrogen	2	0·041	0·046	0·049	0·038
	7	0·040	0·051	0·068	0·052
No nitrogen	3	not available	0·107	0·164	0·084
	8	0·049	0·074	0·135	0·095

1927 was a year of abnormal rainfall, as much as 17 in. falling in the period April to September. In the two dry years, 1928, 1929, the proportion of weeds increased considerably on all plots and fell again in the wet year 1930. On Plot 2 the ratio in 1930 was a little lower than in 1927, on Plot 7 appreciably higher. On the no-nitrogen Plot 8 the weeds were proportionately twice as plentiful in 1930 as in 1927, and in the dry season, 1929, nearly three times. There is, therefore, some evidence that the rotational grazing itself favours the development of "weeds," but that this tendency is largely, if not completely, counteracted by the application of nitrogenous manures.

CHEMICAL RESULTS.

Dry matter content of the separated constituents. Since the separated "grasses," "clovers," "weeds" and "waste" were weighed both before and after drying, the dry matter percentages were obtained: the annual averages for the plots are given in Table VIII.

Table VIII. *Percentage dry matter in separated constituents. Annual averages.*

Year	Plot	Grasses	Clovers	Weeds	Waste	Plot	Grasses	Clovers	Weeds	Waste
1928	2	25·48	24·59	19·59	59·15	3	28·00	24·48	16·95	60·63
	7	26·34	21·88	15·98	49·41	8	27·55	25·70	18·78	57·95
1929	2	27·36	27·85	21·27	49·50	3	33·09	29·20	22·33	65·10
	7	31·92	28·82	20·13	60·63	8	34·28	29·30	24·95	62·27
1930	2	20·80	19·72	16·83	41·77	3	23·22	18·70	15·02	49·77
	7	23·73	20·00	16·10	37·00	8	26·50	20·82	17·50	51·73

	Grasses	Clovers	Weeds	Waste
Average control plots ...	28·77	24·80	19·25	57·91
Average nitrogen plots ...	25·94	23·81	18·31	49·57
Difference	2·83	0·99	0·94	8·34

The averages for the three years show that the effect of the nitrogen is to lower the percentage of dry matter in all the constituents. Although these figures are based on as many as twenty determinations, the differences shown cannot be regarded as accurately measured since the plots are sampled on different dates and therefore under varying weather conditions. The significance of the results is confirmed, however, by the following figures obtained in 1929 on one of the "Latin squares" already mentioned, where such variations were eliminated by taking all the samples on the same day at the same hour.

Percentage dry matter in

	Grasses	Clovers	Weeds
No-manure plots	28·7	29·0	21·9
K + P plots	29·0	26·75	22·1
K + P + N plots	24·4	25·0	17·9
No. of tests	7	5	7

In this series the dry matter in the grasses, clovers and weeds is reduced by about 4 per cent. All the figures show the great importance of estimating dry matter whenever nitrogen-treated plots are being compared with others.

Nitrogen content of the separated constituents. Throughout the investigations nitrogen determinations have been made on the groups of constituents and also on certain of the grass species. Since the system of separation places most of the soil into the section "waste" and the degree of contamination varies greatly, the nitrogen figures so obtained are of greater value and interest than those based on the gross herbage.

Following the usual practice, the nitrogen content has been expressed as crude protein ($N \times 6.25$). The detailed results for each grazing round are not given but have been used to calculate from Table III the *weight* of protein produced, and from these, in turn, annual *weighted* percentages have been compiled. These are given in Tables IX and X.

From the former of these tables it will be seen that clovers are invariably superior to grasses or weeds in protein content, weeds as a rule and on the average coming second. Putting together all the plots for the three years, the relative protein content has been: grasses 100, weeds 107, clovers 145. (If unweighted averages are used instead of weighted ones the relative figures are practically the same.) It is evident, therefore, that botanical composition of the herbage may have an important influence on protein content.

The effect of the nitrogen on grasses and weeds is very similar, producing an additional 2·39 per cent. of protein in the former and 2·56

Table IX. *Annual weighted percentages of crude protein in dried constituents.*

Year	Grasses	Clovers	Weeds	Waste	Total	Net total	Grasses	Clovers	Weeds	Waste	Total	Net total
		Plot 2 (nitrogen)							Plot 3 (control)			
1928	18·66	24·57	19·64	10·48	17·44	18·85	16·58	25·29	19·28	9·65	16·06	17·41
1929	18·47	23·71	18·76	8·97	16·81	18·56	15·36	25·66	15·19	7·07	13·91	16·25
1930	19·33	31·28	19·14	5·14	17·36	19·34	15·28	25·25	18·19	4·81	14·68	15·96
Av.	18·82	26·52	19·18	8·20	17·20	18·92	15·74	25·40	17·55	7·18	14·88	16·54
		Plot 7 (nitrogen)							Plot 8 (control)			
1928	17·54	22·90	20·73	10·43	16·10	17·76	17·40	24·00	18·34	8·02	15·86	17·81
1929	15·71	24·36	17·12	8·53	13·79	15·92	12·44	20·00	12·94	6·62	11·63	13·27
1930	17·39	24·25	19·92	7·52	16·60	17·64	15·96	24·48	16·30	4·86	14·52	17·06
Av.	16·88	23·84	19·26	8·83	15·50	17·11	15·27	22·83	15·86	6·50	14·00	16·05

Weighted percentages for the three years.

	Grasses	Clovers	Weeds	Waste	Total	Net total
Nitrogen plots	17·94	24·40	19·18	8·40	16·35	18·06
Control plots	15·55	24·07	16·62	6·55	14·45	16·31
Difference	2·39	0·33	2·56	1·85	1·90	1·75

Comparison between dry and wet year. Percentage crude protein in dry matter.

	1929 (dry)	1930 (wet)	Difference
Nitrogen plots	15·12	16·98	1·87
Control plots	12·76	14·60	1·84
Difference	2·36	2·38	—

in the latter, when the plots are averaged for the three seasons. In the case of clovers the effect is small, viz. 0·33 per cent.; this figure, though small, can be shown statistically to be significant.

From these results it can readily be deduced that an untreated plot containing 30 per cent. by weight of clovers and 70 of grasses and weeds would have approximately the same percentage of crude protein as a nitrogen-treated plot containing no clovers but only grasses and weeds, the amount of weeds in both cases being assumed not to exceed 15 per cent. of the total herbage. Such a high content of clovers as 30 per cent. *by weight* is probably uncommon on old pastures but is attainable on new ones.

By combining the average dry matter percentages of Table VIII with the average protein content given in Table IX, the crude protein in the herbage constituents *as cut* can be estimated.

Crude protein percentages before drying.

	Grasses	Clovers	Weeds
Nitrogen plots	4·65	5·80	3·51
Control plots	4·47	5·97	3·21

Since the clovers are more important on the control plots, the gross herbage on these, as grazed, will be approximately as rich in protein as that on the nitrogen-treated plots.

The variation of protein percentage with period of the year is well shown by averaging the figures for the plots at the successive grazing rounds, making use of those for the chief constituent, grasses (in 1928 the third and fourth grazing rounds are put together).

Grasses: variation of crude protein percentages with period of year.

	1928	1929	1930	Average
Average all plots:				
Round 1 (May)	20·39	19·64	20·82	·20·28
Round 2 (June–July)	14·91	14·21	14·09	14·40
Round 3 (September)	19·93	15·22	18·20	17·78
Average	18·41	16·36	17·70	—

In each year there is a considerable fall in the protein percentage between the first and second rounds and a smaller rise in the third. As mentioned later the estimated average age of the grass in the first round has been 30 days and in the second, 36 days. So large a fall in the second round cannot, therefore, be simply a question of age but must be attributed to the normal tendency of the grass at this period to hasten towards maturity. Under purely experimental conditions this tendency, leading to a drop in the protein percentage, could be counteracted to a considerable extent by increasing the intensity of stocking, so that grazing took place at a younger stage, say when three weeks old. Under practical conditions it may be very difficult to achieve this, particularly when the spring "flush" comes with unusual rapidity, or it may actually be undesirable, on economic or other grounds, to do so.

The same figures illustrate the effect of the dry season 1929 as compared with the wetter years 1928 and 1930. In the third grazing round of 1929 there is no recovery to the high figure of about 18 to 20 per cent. of crude protein found in 1928 and 1930.

Since in each year there was a very light preliminary grazing with sheep in March (which has not been taken into account in the chemical and botanical records) it is possible to estimate the age of the grass when sampled at the first complete grazing round. This has averaged 30 days during the three years and has not varied much in the individual seasons. For the second round a fairly accurate estimate can also be given, since the grass recovers quickly from the first grazing: the average has been 36 days. For the third grazing round, though the time from the end of the previous grazing could be taken as the age of the grass,

this would be extremely misleading in a dry season. For example, in 1929, owing to the prolonged drought, growth practically ceased for a long time and a period of eleven weeks elapsed from the end of the second grazing to the beginning of the third; even then, as will be seen from Table III, the amount of herbage present was very small. It would, therefore, be incorrect to regard eleven weeks as the age of the grass sampled in September, 1929.

Taking the results as a whole they suggest that, in the spring, month-old "grasses" contain about 20 per cent. protein in the dry matter: in June, when there is the normal tendency for the grass to mature, the figure will be about 14 per cent. for five-weeks-old samples: in the late summer, if there is sufficient moisture for growth, month-old grasses will again reach 20 per cent.

For younger samples, especially in a period of active growth, much higher figures may be obtained. On the "Latin square" previously referred to, "grasses," separated from samples cut in October, 1929, three weeks after the rain which ended the drought of that year, had a protein content of 26·7 per cent. in dry matter on the no-nitrogen plots and 31·95 on the sulphate of ammonia plots. These are the highest

Table X. *Total weight of crude protein (cwt. per acre).*

Year	Grasses	Clovers	Weeds	Waste	Total	Grasses	Clovers	Weeds	Waste	Total
		Plot 2 (nitrogen)					Plot 3 (control)			
1928	4·33	0·135	0·21	0·53	5·205	3·70	0·45	0·46	0·535	5·145
1929	4·82	0·09	0·24	0·56	5·71	2·615	0·50	0·425	0·53	4·070
1930	9·21	0·10	0·35	0·43	10·09	6·18	0·54	0·62	0·29	7·630
Total 3 years	18·36	0·325	0·80	1·52	21·005	12·495	1·49	1·505	1·355	16·845
		Plot 7 (nitrogen)					Plot 8 (control)			
1928	4·59	0·08	0·28	0·85	5·80	2·82	0·24	0·22	0·37	3·65
1929	4·42	0·09	0·33	1·05	5·89	2·21	0·46	0·31	0·49	3·47
1930	8·33	0·24	0·50	0·44	9·51	5·16	1·26	0·50	0·52	7·44
Total 3 years	17·34	0·41	1·11	2·34	21·20	10·19	1·96	1·03	1·38	14·56

Average annual production of crude protein (cwt. per acre).

	Grasses	Clovers	Weeds	Waste	Total	Ratio
Nitrogen plots	5·950	0·122	0·318	0·643	7·033	134
Control plots	3·781	0·575	0·422	0·456	5·234	100
Difference	2·169	−0·453	−0·104	0·187	1·799	—

Comparison between protein production in dry year (1929) and wet year (1930).

	Control plots		Nitrogen plots		All plots	
	1929	1930	1929	1930	1929	1930
Cwt. per acre	3·77	7·535	5·80	9·80	4·785	8·67
Ratio	100 :	200	100 :	169	100 :	181

figures obtained for "grasses" in the course of four years' work: for "clovers" the maximum has been 36 and for "weeds" 29·37, these also occurring on nitrogen-treated plots of the "Latin square."

Total production of crude protein. Table X shows that over the three years the nitrogen plots have produced 34 per cent. more protein than the controls, and that this increase was due mainly to the grasses: the greater weight of clovers on the controls has tended to reduce the difference. In the dry season, 1929, the increase was 54, and in the wet one, 1930, 30 per cent.

The very much greater production of protein in a wet year is shown at the bottom of the table. On the controls the wet year just doubles the protein production of the dry year: on the nitrogen plots the difference is 69 per cent. As in the case of dry matter yields, rainfall is clearly the dominating factor.

Recovery of nitrogen applied. If the average gross difference of 1·799 cwt. per acre of crude protein shown in Table X is assumed to be an accurate measurement of the extra protein produced by the nitrogen plots, this would contain 0·288 cwt. of nitrogen as compared with 0·618 cwt. applied in the 3 cwt. sulphate of ammonia. This would be a recovery of approximately 46 per cent.

Crude protein content of the chief grass species. The figures given in Table XI are of special interest in view of the changes in the proportion of certain grass species which are proceeding on the plots. They are of interest also in that the species analysed were growing together in competition on large grazed plots and were not grown pure in small plots under more "artificial" conditions.

Sheep's Fescue and Bent Grasses have consistently been richer in crude protein than Perennial Rye Grass on both nitrogen and control plots. In 1928 Meadow Grasses were below Perennial, but in 1930 slightly above; Yorkshire Fog has been superior to Perennial in the two years 1929 and 1930. It is clear from these figures that an increase in the proportion of Sheep's Fescue and Bent Grasses, whatever effect it may have on the total weight of herbage produced, will tend to increase the percentage of crude protein in the herbage as grazed.

The superiority of Sheep's Fescue and Bent Grasses over Perennial Rye Grass is probably to be accounted for by the method of sampling. The first is a dwarf species and the second a creeping one, so that cutting by shears would tend to include in the sample a greater proportion of leaf with these two species than with Perennial Rye Grass, which is upright in habit.

Table XI. *Percentage crude protein in the chief grass species*
(dry matter basis).

	1928			1929			1930		
Grazing round	Nitrogen plots	Control plots	Difference	Nitrogen plots	Control plots	Difference	Nitrogen plots	Control plots	Difference
				Lolium perenne					
1	20·30			22·35	18·80	3·55	21·68	16·36	5·32
2	15·75	15·25	0·50	11·62	10·24	1·38	15·11	11·74	3·37
3	26·80	21·90	4·90	16·29	15·75	0·54	19·73	19·56	0·17
4	20·60	18·95	1·65	—	—	—	—	—	—
Average	21·05	18·70	2·35	16·75	14·93	1·82	18·84	15·89	2·95
Average of all	19·98			15·84			17·36		
				Agrostis spp.					
1	26·80			26·50	24·27	2·23	27·50	21·85	5·65
2	19·25	17·95	1·30	18·24	15·74	2·50	17·68	15·66	2·02
3	21·20	18·60	2·60	16·38	15·96	0·42	20·14	19·88	0·26
4	23·50	19·40	4·10	—	—	—	—	—	—
Average	21·31	18·65	2·66	20·37	18·65	1·72	21·77	19·13	2·64
Average of all	21·69			19·51			20·45		
				Festuca ovina					
1	22·45			21·96	19·43	2·53	23·33	19·22	4·11
2	16·85	17·65	−0·80	16·56	15·47	1·09	18·17	15·01	3·16
3	29·00	25·50	3·50	17·06	14·85	2·21	17·38	17·24	0·14
4	23·30	20·40	2·90	—	—	—	—	—	—
Average	23·05	21·18	1·87	18·53	16·58	1·95	19·63	17·16	2·47
Average of all	22·20			17·56			18·40		
				Poa pratensis and *trivialis* (chiefly *triv.*)					
1	19·70			Not determined			22·25	18·22	4·03
2	14·65	15·70	−1·05	,,			14·78	12·82	1·96
3	16·55	13·95	2·60	,,			18·16	19·09	−0·93
4	18·80	17·10	1·70	,,			—	—	—
Average	16·66	15·58	1·08				18·40	16·71	1·69
Average of all	17·02						17·55		
				Holcus lanatus					
1	Not determined			24·75	20·5	4·25	23·00	19·60	3·40
2	,,			14·70	14·4	0·30	14·75	14·69	0·06
3	,,			16·15	15·0	1·15	18·95	19·44	−0·49
Average	...	...	...	18·53	16·63	1·90	18·90	17·91	0·99
Average of all	...	...		17·58			18·40		

N.B. In Round 1, 1928, samples of a species from all the plots were mixed together: subsequently they were kept separate for the nitrogen and control plots.

Over the three seasons the average increase in the percentage of crude protein produced by the sulphate of ammonia has been in the case of Perennial Rye Grass, 2·37, of Bent Grass, 2·34, of Sheep's Fescue, 2·10. These figures agree fairly closely with that of 2·365 for the "grasses" as a whole.

GENERAL DISCUSSION.

The observations on the rotational system at "Oaklands" have now proceeded for four years, 1927–1930 inclusive; since the first year they have been conducted on a uniform plan. Those years, meteorologically, have been very different, the rainfall, for example, varying from 17·75 to 33·95 in. in a harvest year. The grassland on which they were conducted had been raised previously to a fairly high productive level by thorough cultivation, careful grazing and phosphatic manuring: this level was estimated at two-thirds of a cow per acre for the grazing season. During the four years the grazing capacity has been about four-fifths of a cow per acre on the control plots and one cow on the nitrogen plots. These cow-day figures agree on the average with those obtained by sampling the herbage.

The combined observations give no reason to suppose that the productivity of the grass can be raised to an appreciably higher level by the methods so far employed. Lengthening the period between grazings might increase slightly the total output of dry matter, but would lower its digestibility and protein content: shortening the period would decrease the total output of dry matter but increase its protein percentage and, perhaps, its digestibility. The possibilities of increasing the value of the output per acre are, therefore, very limited unless resort is had to such drastic measures as ploughing up and re-seeding with a modern "seeds" mixture, or to irrigation.

It should be emphasised that the observations recorded were not intended to measure the effect of the rotational system of grassland management as a whole, but only that part which may be attributed to the nitrogenous manures. So far as the other factors are concerned—the dense stocking, the repeated harrowings, etc.—the only measure of their effect is the difference between the two-thirds cow per acre estimated as the grazing capacity before 1927 and the four-fifths during the past four years on the no-nitrogen plots. Evidently at "Oaklands" the influence of these factors has been small, as would have been anticipated since the prior improvement due to management had been at least 100 per cent.

The conclusion seems irresistible that in Hertfordshire, with an average rainfall of about 26 in., the possibilities of increased output by the system of rotational grazing combined with nitrogenous manuring are very limited in the case of old-established grass which has been well managed. If the system were applied to grass previously badly managed

the apparent improvement might be large, but the whole of the improvement could not rightly be attributed to the features peculiar to that system.

Summary.

An account is given of three years' observations on plots of permanent grass managed according to the "New System of Grassland Management," which consists essentially of periodic dressings of nitrogenous manures followed by rotational grazing.

Results obtained by keeping records of the grazing provided and by sampling the plots show that the dominating factor on total yield has been rainfall. In the wet season 1930 the output of dry matter was about 80 per cent. more than in the dry year 1929: the extra grazing was about 46 per cent.

The increased grazing due to the nitrogenous manures (three dressings per annum each of 1 cwt. sulphate of ammonia per acre) has, on the average, been estimated at between 20 and 30 per cent. above the controls. This increase, however, has not been accurately measured.

It is shown conclusively that the leguminous species have been suppressed on the nitrogen plots and are now not more than one-fifth of those on the controls.

The effect of the "system" on the grass species has been to cause an increase of Sheep's Fescue on all plots. Meadow Grasses and Yorkshire Fog have decreased everywhere: Rye Grass has either maintained an already high position or has considerably increased. Five grass species account for 80 to 90 per cent. by weight of "grasses" on all plots. There is no evidence of differential effect due to nitrogen.

The rotational grazing has apparently tended to cause an increase in "weed" species, but this has been, at least partially, counteracted by the nitrogen dressings.

The crude protein content of herbage components grouped under "grasses," "clovers" and "weeds" has been determined for the nitrogen and control plots. The nitrogen has increased protein percentages in all three groups, the average effect being to give an additional 2·39, 0·33 and 2·56 per cent. of crude protein respectively in the dried constituents.

In spring, "grasses" at an age of four weeks have about 20 per cent. crude protein: in early summer at an age of about five weeks this falls to about 14·5: in a wet season it may rise to 20 or even more in the late summer, but will fail to reach this figure in a very dry year. "Clovers" on an average have about 45 per cent. more protein than "grasses":

"weeds" are only about 7 per cent. richer. Botanical composition may, therefore, have a big influence on the protein content of pastures.

Figures are given showing the protein content of certain individual grass species. Sheep's Fescue and Bent Grass have consistently been higher than Perennial Rye Grass.

A general conclusion of four years' experience of the "New System" in Hertfordshire is that the possibilities of additional output by its application to well-managed permanent grassland are limited to the 20 or 30 per cent. increase which has been obtained: if, however, it were applied to poorly managed grass the improvement might be much greater.

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<div align="center">*(Received May 10th, 1931.)*</div>

THE VALUE OF "STICKY POINT" DETERMINATIONS IN FIELD STUDIES OF SOIL MOISTURE.

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(With One Text-figure.)

THE great local variation in the soil moisture under field conditions is very troublesome to those who investigate questions involving the evaluation of the soil moisture, and·necessitates a large amount of sampling-to reduce as far as desirable the error due to this cause. Variations in soil texture are responsible for much of this variation, so that a method that will correct this to any appreciable extent is very valuable. Comparisons on the basis of "single value" soil constants are the most suitable for this purpose.

Keen and Coutts[1] and later Coutts[2] investigated many suitable constants. Examples of uses of single value constants to correct for soil texture variations in moisture determinations are to be found in the use of the hygroscopic coefficient by Batchelor and Reed[3] and the moisture equivalent by Conrad and Veihmeyer[4]. In both cases the authors expressed the moisture content as a ratio of the single value constants employed. The hygroscopic coefficient is often used by plant ecologists to compute the soil water available to the plant.

Puri[5] has shown objections to the use of the hygroscopic coefficient, but suggests the use of the moisture content at 50 per cent. humidity, while the facilities for determining the moisture equivalent are not available to all laboratories.

Hardy[6], in investigating certain soil moisture constants, measured the "total bound water" by determining the moisture content when the plastic soil mass just adhered to external objects, and the writer has found this constant, *i.e.* the "sticky point," convenient for use in correcting for variations in soil texture in field studies of soil moisture, using the technique described by Keen and Coutts[1].

To investigate the variability of the soil moisture under field conditions, samples were taken from a field in bare fallow, eight days after an inch of rain had fallen. Prior to this fall the soil was quite moist owing to previous rains. The soil profile is as follows: a surface loam

0–20 cm., a clay band 20–40 cm., below 40 cm. limestone nodules begin to appear, and the clay assumes a lighter colour. A sharp line of demarcation occurs between the surface loam and the clay. The clay from 20–40 cm. appears to the eye to be very uniform in texture.

A pit of suitable size to permit ease of working was dug, and the northern side was trimmed square. Skewers were pushed into the soil face in appropriate positions to serve as guides in sampling. Fifty samples of approximately 50 gm. (dry weight) were taken from the face in the clay in two rows 30 and 35 cm. respectively from the surface, the samples being taken from points 5 cm. apart in the rows. A few inches of soil was removed from the face immediately before sampling, and other appropriate precautions taken to ensure samples that would show accurately the moisture content in the field. If the texture of the soil were uniform, such samples should show the same moisture content.

The moisture content was determined by drying at 105° C. for 48 hours, after which the samples were ground and sieved and duplicate sticky point determinations made upon the samples[1].

Considering first the samples taken at 30 cm. and those at 35 cm., the following results were obtained:

	Mean moisture content (%)	Mean sticky point (%)
Top row (30 cm. from surface)	24·12	22·83
Bottom row (35 cm. from surface)	27·87	26·10

It is seen that the soil contained more moisture at a depth of 35 cm. than at 30 cm.; moreover, the results show that this was consistently the case in each of the twenty-five pairs of samples from the 30 and 35 cm. depths respectively. However, similar results were found with the sticky points, and from the similarity of the following equations connecting the moisture content with the sticky point, it must be assumed that the difference in moisture content noted was entirely due to differences in the physical nature of the soil[2]. The relationship between the two values can be expressed by the following equations:

$$M = 0.72S + 7.48 \quad \text{for top row,}$$
$$M = 0.78S + 7.73 \quad \text{for bottom row,}$$

where M = moisture content in the field and S = sticky point.

In this particular case the moisture content of the soil in the field

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[1] Agreement between duplicates was usually good. In eight cases a difference of more than 0·6 was obtained, and the determinations were repeated.

[2] *I.e.* differences in texture or perhaps humus content.

was in the neighbourhood of the sticky point. A second test was therefore made of the same soil horizon in a neighbouring field and an appreciable time after rain had fallen, where grass had dried out the soil considerably. In this case sixty samples were taken. The results of both experiments are plotted in Fig. 1.

In an experiment to determine the relative evaporation from the soil when it was cultivated and when left undisturbed, samples were taken thirty days and twenty-three weeks respectively after a heavy irrigation had been applied but with no subsequent rain or irrigation water. No weeds were permitted to grow during the interval(7).

The following table summarises the results of the four sets of samplings:

Sampling	No. of samples	Mean moisture content (%)	Mean "sticky point" (%)	r	c	k $(S - M)$
Fallow, 8 days after rain (Fig. 1, crosses)	50	25·99	24·47	+0·950	0·871	−1·52
Grass soil dried out considerably (Fig. 1, circles)	60	19·55	30·60	+0·878	0·748	11·05
30 days after irrigation	24	25·85	27·50	+0·860	0·813	1·65
23 weeks after irrigation	24	24·17	27·97	+0·912	0·955	3·80

In the third and fourth columns the mean field moisture contents and mean sticky points are given respectively, in the fifth column the correlation coefficients between the field moisture contents and sticky points are given, while in the sixth column the regression coefficients of M on S are given, and in the seventh column the difference between S and M are shown. For the first two sets of samples these relationships are illustrated in Fig. 1. In each case a high positive correlation exists between M and S.

Assuming, therefore, a linear relationship between the moisture content of the soil and any given degree of moistness, the following equation would hold:

$$M = bS - K,$$

where b is the regression of the moisture content on the sticky point.

For many purposes the simpler relationship $K = S - M$ is sufficiently exact for practical purposes of comparison.

The water content of the soil shortly after an irrigation appears more or less constant for any particular soil, but varies quite considerably with the texture of different soils, and has been variously called the field moisture capacity(8), normal moisture content(9), and maximum molecular moisture holding capacity(10). From the above data in the

soil studied this appears to be about the sticky point of the soil, and this has been found to be the case, as a general rule, with local soils[1].

If the moisture content is above the sticky point, sampling is impracticable, and in fact, the water has not adjusted itself to approximate equilibrium conditions.
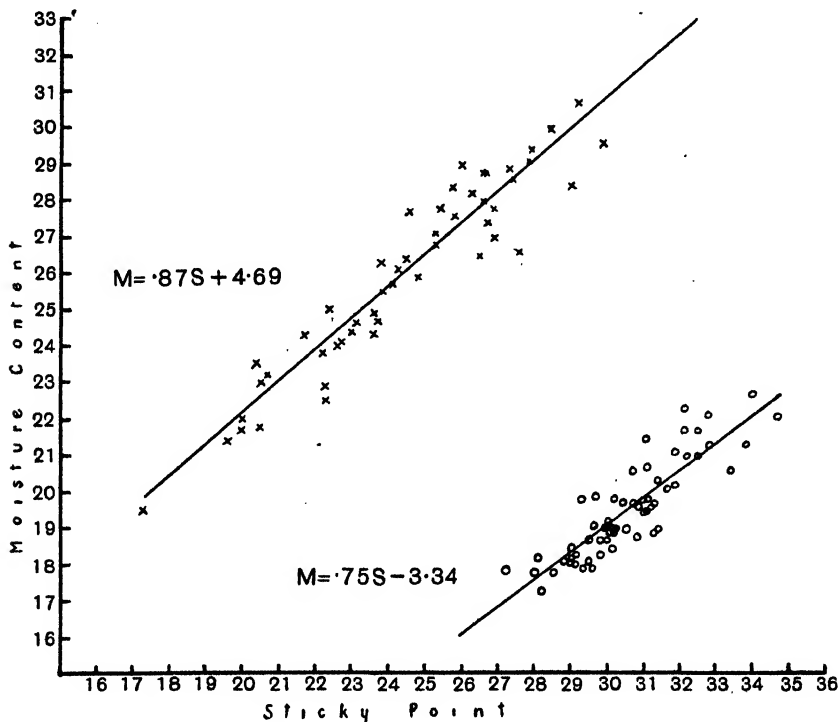

Fig. 1 Illustrating relationship between field moisture contents and sticky points of two series of soil samplings.

In this case, then, the constant k in the above equation represents the amount of water that must be added to moisten the soil again, or the amount that the soil has dried out since the last irrigation. The assumption that the regression of the moisture content on the sticky point is unity implies that the amount of water lost by evaporation or

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[1] Israelson (11) and Shaw (9) found the field moisture capacity to approximate closely to the moisture equivalent, while Hardy (6) suggests that the sticky point is above the moisture equivalent. From this one would expect the sticky point to be above the field moisture content, but experience with local soils does not bear this out.

transpiration after a given time is constant throughout the soil considered.

As illustrative of the gain in precision in making the above correction of subtracting the observed moisture content from the sticky point, the following example is given.

Samples are taken at five depths weekly near eight orange trees. The standard deviations within the individual samplings of the actual moisture contents and of the differences between the sticky points and moisture contents for the eighty samples of each depth after ten samplings were as follows:

| | | Standard deviations | |
Depth	Moisture content	Difference between moisture content and sticky point
10–20 cm.	1·45	0·85
20–40 „	3·83	0·92
40–60 „	2·40	1·05
60–80 „	2·77	1·32
80–100 „	2·85	2·17

The depth 10–20 cm. is the surface loam. The first 10 cm. is discarded, as it represents mainly the loose cultivated soil.

A considerable gain in precision is obtained for each depth. The surface soil is comparatively uniform in texture, and the error due to soil texture variations is relatively small; nevertheless, considerable gain in precision is obtained by using the correction. Although the clay band appears uniform in texture, a rather large variation occurs and a considerable gain in precision is obtained. To obtain the same gain in precision in determining the mean moisture content, the number of samples taken would have to be increased fifteen fold. The gain in precision with lower depths is not so marked, due to the fact that limestone nodules occur with increasing frequency, and although they reduce both the moisture content and sticky point, the reduction is by no means proportional; nevertheless, even for the depth 80–100 cm. the gain in precision is equivalent to doubling the number of samples taken.

It is to be noted also that the sticky point determinations are made on the actual samples used for the moisture determination, thus eliminating the further sampling error that would be involved if a separate sample were taken for the sticky point determination, or if the sample from the field were divided into two portions, one to be used for the moisture determination, and the other for the sticky point determination. Should, therefore, the heating of the soil to 105° C. affect its sticky point, the sticky point so determined will not be identical

to the sticky point of the fresh sample. As, however, all samples will be similarly affected, the value of the correction will be in no way impaired.

In order to gain some information as to the possible effect of oven drying, fifteen determinations were made on air-dried and on oven-dried bulk samples of four different local soils, with the following results:

Description of soil	Mean sticky point			t	P	Greatest possible real difference
	Air-dried	Oven-dried	Difference			
Surface loam, 0–20 cm.	13·653	13·293	0·360	2·17	<0·05	0·7
Clay subsoil, 20–40 cm.	32·473	32·807	− 0·334	0·98	0·3	0·4
Clay subsoil, 60–80 cm., rich in lime	30·087	29·473	0·614	2·50	·0·02	1·0
Grey self-mulching clay*	31·307	31·036	0·271	1·64	>0·1	0·6

* Only 14 determinations made of each of the air-dry and oven-dry samples.

The soil of the depth 60–80 cm. contains a large amount of limestone nodules in the field, and the prepared sample contains a high proportion of particles of limestone that pass a 2 mm. sieve.

In the fourth column the difference sticky point of air-dry soil—sticky point of oven-dry soil is given. In the fifth and sixth columns the ratio t and probability P of Fisher's table of t are given[1]. The difference of 0·36 per cent. between the means for the surface soil is probably significant, while that of 0·614 of the 60–80 cm. sample is certainly significant. In the other two cases the differences are not statistically significant.

It is of interest to know what the extreme possible differences in the true means of the air-dried and oven-dried samples would be, and this is shown in the seventh column[2]. With these soils, therefore, oven-drying evidently has in some cases a little influence on the sticky point, but this influence cannot be very great.

It is possible, of course, that larger differences may occur in other types of soils, perhaps, for example, in humus soils.

Experience in making sticky point determinations seems to indicate that with some soils a very satisfactory degree of precision is obtained, but in others the precision is not so good; but excepting very sandy soils, the errors involved in the sticky point determinations are small compared with the sampling error due to soil heterogeneity in the field. As pointed out by Hardy and by Keen and Coutts, with very sandy soils the sticky point loses its significance. The wet sample is

[1] *Statistical Methods for Research Workers* (1928), R. A. Fisher. Oliver and Boyd.

[2] In the case of the 20–40 cm. depth sample it is assumed that if any difference occurred the sticky point of the air-dry sample would be greater than that of the oven-dry sample.

not plastic and has no sticky point in the same sense as sandy loams, loams, or clays.

SUMMARY.

In comparing the moisture contents of soils in the field, material precision is to be gained if the sticky points are also determined and the comparison based on the difference between the two values. There is a high degree of correlation between the moisture contents and sticky points of soils in moisture equilibrium with each other.

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STUDIES OF THE SULPHUR OF PASTURE GRASS.

I. THE CYSTINE CONTENT OF PASTURE GRASS.

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INTRODUCTION.

THE nutritional significance of the amino acid cystine was clearly demonstrated by Willcocks and Hopkins and later by Osborne and Mendel. Subsequently, several investigations have been made with other sulphur-containing organic bodies which are closely similar to cystine in structure, so as to find whether these compounds are capable of transformation into cystine in the animal body. The result of all these investigations has been negative. It is, therefore, generally considered that cystine must be present pre-formed in the food.

This conclusion is of considerable importance, and has an important bearing on the feeding of sheep, since wool keratin has been shown to contain 13 per cent. of cystine[1]. The requirements of sheep for this sulphur-containing amino acid is, therefore, fairly high.

Practically all proteins contain sulphur, but according to the recent work of Sullivan[2] the proportion of the sulphur present as cystine may be almost negligible. Thus, in the case of phaseolin, the main protein of the navy-bean (*Phaseolus vulgaris*), the presence of cystine is very doubtful. Only one other sulphur-containing amino acid has been isolated from protein hydrolysates, namely, methionine ($C_5H_{11}SNO_2$)[3], the sulphur of which is completely oxidised to sulphate in the animal body.

Investigations on the cystine content of foodstuffs have been carried out by two distinct methods; firstly, by the precipitation and estimation of the amino acid gravimetrically or directly by means of colorimetric methods or, secondly, by feeding trials in which the growth increment has been compared with and without the addition of cystine to the basal diet. Where the amount present is small, the use of colorimetric methods of estimation have proved to be both useful and convenient. Two outstanding methods have been used in this respect; the Folin-Marenzi[4] reagent, which gives a blue colour with cystine, and the Sullivan reagent[2], which gives a bright red colour. The former has been

criticised on the ground that it is not specific for cystine but that it gives a similar colour with other disulphides as well as with pyruvic acid and furfural [2]. That interfering substances do occur seems to be supported by this work. The Sullivan reagent, however, seems to be specific for free cysteine or cystine on reduction.

The second method of determining cystine deficiency has already been applied to pasture grass by Robertson and his associates in Australia [1]. Robertson worked on the supposition that cystine was the limiting factor in wool production under Australian pastoral conditions. He, therefore, carried out experiments to determine the influence of added cystine on the fleece weight and sulphur content of the wool. The results obtained during 1927–28 were negative. This was attributed by Robertson, among other things, to the fact that "the pasture upon which the animals were grazing was itself too rich in cystine to permit the relatively small addendum of cystine to make any perceptible difference." The following season, however, by controlling the basic diet and ensuring its deficiency in cystine, strikingly positive results were obtained. As a result of this and later work with lambs, the Australian workers have advocated the feeding of blood meal (3 per cent. cystine) to sheep on their pastoral areas. Whether the beneficial effect of feeding blood meal is to be attributed to its cystine content or to the addition of protein of high biological value is a question on which further information is desirable.

In view of the fact that pasture grass, in the majority of cases, forms the sole diet of sheep, particularly in the sheep-rearing districts, it is surprising that so little information is available on the organic sulphur and cystine content of British pastures. On the other hand, no evidence has been brought forward to suggest that the sulphur content of the pastures is a limiting factor in either growth or fleece production. Recently, however, an important contribution has been made to this subject by Aitken [5] working in New Zealand. Aitken worked on the leaves of five species of pasture grass, namely, Yorkshire fog, cocksfoot, perennial rye grass, prairie grass and hair grass. He found that the worthless grasses were as rich in organic sulphur as the valuable species like cocksfoot and perennial rye grass, which indicates that the botanical character of the herbage is no guide to its cystine content. Further, after weak acid hydrolysis, both of the grass itself and of protein isolated from grass, he attempted, both by means of precipitating reagents and by the Sullivan colorimetric method, to estimate the amount of cystine present. The results obtained were all negative and Aitken concludes

that "there is no evidence in support of the assumption that the organic sulphur of grass is present in the form of cystine." This implies the presence of a cystine precursor in grass.

In this connection the work of Sherman and Woods[6] on the cystine content of casein is also interesting. The experimental method employed was to feed a basal diet deficient in cystine and then to find the relationship between the amount of pure cystine, on the one hand, and of casein, on the other, required to produce a growth of 10 to 17 gm. in six weeks. On this basis they found "that casein contained from 1·3 to 2·5 per cent. of cystine or cystine plus nutritionally equivalent sulphur-containing radicles." This result is ten times higher than the amount of cystine shown to be present by chemical means. This again suggests that some precursor of cystine must be present in casein.

That sheep can produce wool normally after subsisting on poor pastures over indefinite periods and with no supplements is well known. The question of the combinations in which sulphur is present in pasture grass is, therefore, of considerable interest.

The estimation of total sulphur in grass.

Carius's method of estimating sulphur, while no doubt accurate, is unsuitable where a large number of determinations have to be carried out, in that it involves a considerable amount of labour. Further, when the sulphur content of the material is small, a considerable quantity has to be used in order to get a suitable amount of $BaSO_4$ precipitate for gravimetric estimation. The risk of explosion and breakages is, consequently, considerably greater when using a substance like pasture grass.

The estimation of sulphur by oxidation with sodium peroxide in a nickel crucible is also both tedious and very difficult to manipulate. The rapid deterioration of the nickel crucibles and the sudden ignitions which often occur, sometimes with explosive violence in the case of grass, make it imperative that a more convenient method be adopted.

The sodium peroxide oxidation can be carried out with considerable ease, however, by using the Hodsman bomb[7] instead of a crucible. The bomb, which was first designed for the estimation of sulphur in coal, consists of an inner cartridge of steel tube with a tight-fitting cap. One gram of grass is intimately mixed with sodium peroxide and placed in this cartridge. The cap is fitted on and the cartridge is placed in an outer cylinder, the upper part of which screws on to the bottom part. A screw passing through the top of the outer cylinder is then tightened

so as to keep the cap of the inner cylinder firmly in position. The bomb is then placed in a clamp, in an inclined position, and its base is heated· for a few minutes with a bunsen burner until firing occurs. After cooling, the bomb is unscrewed and the cap is removed from the inner cylinder which is then placed in a dish of cold water. The dish is immediately covered with a clock glass so as to prevent loss by spattering while the flux dissolves. The solution, containing the sulphur as sulphate, is then transferred to a beaker and acidified with conc. HCl and boiled. Ammonia is added to precipitate any iron and the solution is filtered. A perfectly clear solution is thus obtained for precipitating the sulphate by means of barium chloride. After standing twenty-four hours the barium sulphate precipitate is filtered through an ignited and weighed Gooch crucible. The crucible is then dried and further ignited with the usual precautions to prevent loss of $BaSO_4$.

To test the accuracy of the method, two samples of grass were analysed for total sulphur using this method as well as the Carius tube and the Benedict-Dennis reagent. The results obtained were as follows:

Percentage total sulphur on dry matter.

	Hodsman bomb	Carius	Benedict-Dennis
(1)	0·62	0·60	0·67
(2)	0·59	0·59	0·64

It will be observed that while the agreement between the Hodsman bomb and Carius methods was very close, the Benedict-Dennis method gave slightly higher results. This is probably due to the difficulty of getting the reagents free of sulphur. The fact that the Hodsman bomb method is both simple and accurate makes it of great value for the determination of total sulphur in biological material, especially where a large number of determinations have to be carried out.

The inorganic sulphur was estimated by igniting 5 gm. of grass in a silica basin until a greyish white ash was obtained. The ash was then extracted with 10 per cent. HCl and an aliquot portion of the filtrate was taken and the sulphate precipitated with $BaCl_2$ as above.

THE BOTANICAL NATURE OF THE HERBAGE.

All the grass samples analysed in the first part of this investigation were obtained from the same field and also from plots that have been under experiment for a number of years in connection with the pasture research carried out at this Institute. The botanical nature of the her-

bage[1] varied considerably during the season, but in the spring samples the chief grasses were perennial rye grass and rough-stalked meadow grass. Towards mid-season there was also a very appreciable growth of wild white clover, especially under the weekly and fortnightly systems of cutting. Later in the season creeping bent became very prominent and towards October formed the major part of the grasses.

The plots were situated on light sandy soil and the pasture, consequently, was only of medium quality. The field had received no dressing of artificial manures for a number of years until 1925 when 10 tons per acre of basic slag were applied.

The samples obtained were first dried on a hot plate and afterwards in a water oven at 100° C. until constant in weight. It is possible that a slight loss of sulphur occurs by volatilisation during the drying process. That this loss is almost negligible in the case of grass, however, is shown by the results of Peterson(8), who found that with June grass the loss by volatilisation was only 0·002 per cent., while with green clover it varied between 0·004 and 0·014 per cent. sulphur. Peterson suggests that this sulphur is probably of sulphide form.

THE INFLUENCE OF MATURITY ON THE SULPHUR OF PASTURE GRASS.

Recent work on pasture grass has shown that as the grass becomes mature there is a distinct decrease in the percentage of both crude and true protein. An extreme example of this is the difference in the protein content of week old herbage and hay. If the organic sulphur is present in the protein as cystine, one would, therefore, expect to find a direct correlation between the organic sulphur and protein content. To investigate this point, samples of grass were obtained at the weekly, fortnightly, monthly and early hay (flowering) stage of growth. The results obtained are given in Table I.

It will be observed that there is no difference in total sulphur content between the grass cut weekly and that cut every fortnight, but that the monthly cut grass shows a lower average while the hay samples were distinctly poorer in total sulphur. These variations are interesting in that they are somewhat similar to those observed in the case of the protein content. The average crude protein content of the weekly cut grass was 23·72 per cent. (20·8 per cent. true protein), in the monthly cut grass 18·54 per cent. (16·6 per cent. true protein), while in the hay samples the amount of crude protein was 11·0 per cent. (10·0 per cent.

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[1] For further details see Woodman, *et al.*, *Journ. Agric. Sci.* (1929), **19**, 236.

true protein). The decrease in both total and organic sulphur between the week-old and hay stage was 38 per cent., while the decrease in crude protein was 53 per cent.

Table I. *Showing the effect of maturity on the total, inorganic and organic sulphur of pasture grass (dry matter basis).*

Composite samples of	Total sulphur (%)	Inorganic sulphur (%)	Organic sulphur (%) (by difference)
(1) Weekly cut grass (1928):			
Apr. 19–May 10	0·64	0·29	0·35
June 14–July 5	0·73	0·38	0·35
July 12–Aug. 2	0·73	0·38	0·35
Aug. 9–Aug. 30	0·62	0·37	0·25
Sept. 6–Sept. 27	0·87	0·39	0·48
(2) Fortnightly cut grass (1928):			
Apr. 26–May 10	0·74	0·28	0·46
June 21–July 8	0·78	0·38	0·40
July 19–Aug. 2	0·75	0·39	0·36
Aug. 16–Aug. 30	0·71	0·34	0·37
Sept. 13–Sept. 27	0·58	0·38	0·20
(3) Monthly cut grass (1929):			
Apr. 20–29	0·53	0·25	0·28
May 14–27	0·62	0·26	0·36
June 8–18	0·72	0·31	0·41
July 13–26	0·66	0·40	0·26
Aug. 11–24	0·63	0·32	0·31
(4) Early cut hay:			
June 1928	0·47	0·22	0·25
June 1929	0·42	0·24	0·18
(5) Poor molinia hay:	0·23	0·12	0·11
Average results under the different systems of cutting.			
Weekly	0·72	0·36	0·36
Fortnightly	0·71	0·35	0·36
Monthly	0·63	0·31	0·32
Hay	0·45	0·23	0·22

It is noteworthy that no correlation was found between the organic sulphur and true or crude protein content of the herbage. This will be evident from the fact that, while the crude protein decreases gradually towards mid-season and then increases, the variation in the organic sulphur showed no definite trend. Further, the amount present in the monthly cut grass was highest in June. This indicates that the organic sulphur must be present in part, at least, in some combination other than protein sulphur. The results also show that the organic sulphur of pasture grass may vary approximately 50 per cent. during the season. This indicates the importance in comparing individual species for organic sulphur content to obtain samples at the same seasonal stage of growth.

If we assume the organic sulphur of the grass to be present as cystine (26·6 per cent. sulphur), the average cystine content of the weekly and fortnightly cut herbage is 1·4 per cent.; that of the monthly cut grass is 1·2 per cent. and that of the early cut hay about 0·8 per cent.

THE INFLUENCE OF HEAVY DRESSINGS OF INORGANIC SULPHATE ON THE ORGANIC SULPHUR OF PASTURE GRASS.

The sulphate sulphur absorbed by the roots from the soil is generally regarded as the source of the sulphur present in the plant in the form of cystine and other sulphur-containing organic bodies. A point of considerable importance, therefore, arises as to whether the application of ammonium or potassium sulphate to a "normal" soil will increase the amount of these essential sulphur compounds present in the plant, or whether the amount of sulphate already present is sufficient to satisfy these requirements. An opportunity for testing this point was presented by a manurial experiment on pasture grass in progress at this Institute (9).

A brief outline of the experiment is as follows. One series of six sub-plots (Plot A) received 2 tons of ground limestone, 5 cwt. of superphosphate and 2 cwt. of sulphate of potash per acre on Jan. 31st, 1929. During the following season (Feb.-Oct.) they received a further application of 4 cwt. of sulphate of ammonia per acre, in seven monthly dressings. On Feb. 15th, 1930, and afterwards at monthly intervals (with the exception of July and September), a further $3\frac{1}{2}$ cwt. of sulphate of ammonia were applied. It will thus be evident that the produce analysed for sulphur content in 1930 had received a heavy dressing of sulphates.

The second series of three sub-plots (Plot B) received only 2 tons of ground limestone and no dressing of inorganic sulphates. On Nov. 8th, 1929, however, both series of plots received 5 tons per acre of well-rotted dung to replace the organic matter which would normally have been returned to the soil in the droppings of the animals grazing on the land.

The soil on which these plots were situated was of a heavy clay type, and the field is reputed to be the best heavy land pasturage on the Cambridge University farm. The results obtained do not, therefore, necessarily apply to poor pastures where probably more pronounced effects could be expected.

In botanical composition, the early spring samples consisted mainly of rye grass, rough-stalked meadow grass and *Agrostis*. Towards June, in addition to the above species, cocksfoot and red fescue were also prevalent. Later in the season *Agrostis* was the dominant grass present.

Of greater interest, perhaps, was the markedly retarding influence of sulphate of ammonia on the weeds and to a less extent on the growth of wild white clover. It was estimated that towards October 95 per cent. of the bulk off the A plot consisted of pure grass species, while on the B plot the produce contained 15–20 per cent. of clover. Apart from this difference, the vegetation on both plots was very similar.

The plots were cut at monthly intervals throughout the season, but the first cutting represents winter and early spring growth. The results obtained for composite samples taken from both plots are given in Table II, together with the protein figures which will be referred to in the discussion.

Table II. *Showing the effect of high sulphate dressings on the sulphur of pasture grass (dry matter basis).*

Date of cutting	Plot	Total sulphur (%)	Inorganic sulphur (%)	Organic sulphur (%) (by difference)	Crude protein (%)
Winter and early spring growth:					
Mar. 23, Apr. 1 and 8	A	0·78	0·37	0·41	25·07
„	B	0·60	0·27	0·33	24·60
Monthly growth:					
Apr. 20 and 29, May 6	A	0·72	0·37	0·35	22·86
„	B	0·57	0·29	0·28	22·38
May 20 and 27, June 3	A	0·88	0·42	0·46	18·67
	B	0·67	0·32	0·35	17·30
June 10, 17 and 24	A	0·91	0·61	0·30	19·66
„	B	0·78	0·46	0·32	20·77
July 15, 22 and 29	A	1·16	0·76	0·40	20·04
„	B	0·93	0·52	0·41	20·64
Aug. 12, 19 and 26	A	1·16	0·68	0·48	19·83
„	B	0·91	0·47	0·44	21·66
Sept. 9, 16 and 23	A	1·14	0·69	0·45	21·05
„	B	0·88	0·47	0·41	22·28
Average results	A	0·96	0·56	0·40	—
	B	0·77	0·40	0·37	—
Monthly growth from light sandy soil (Table I)		0·63	0·32	0·31	—

The application of inorganic sulphates has increased both the total and inorganic sulphur in the grass at all stages throughout the season. The organic sulphur was also increased both at the beginning and end of the season, but in the June and July samples the organic sulphur was slightly higher in the B plots which received no sulphate dressings. The most pronounced increase in organic sulphur was obtained in the May samples, when the A plots showed an increase of 31 per cent. in organic sulphur over the B plots, but the increase in August and September, however, was only 10 per cent. The average results for the total sulphur, throughout the season, show an increase of 25 per cent. in the A plot

over the B plot, but the increase in the organic sulphur was only 8 per cent., showing that high sulphate dressings tend to favour the sulphate sulphur content of the pasture grass rather than the organic sulphur.

The seasonal variation in both total and inorganic sulphur was very similar in both plots, a gradual increase taking place to a maximum content in July and afterwards a very gradual diminution. The variation in the organic sulphur, on the other hand, was much more erratic, a pronounced decrease being obtained in this constituent in June, especially in the herbage of the A plot, compared with the May growth. An apparent explanation of this is obtained from notes taken at the time of sampling. These indicate that the A plots at this period were suffering rather severely from the droughty conditions prevailing at the time, but the B plots, being more protected by the bottom growth of wild white clover, although suffering from drought, were in a much better condition. This point requires further investigation, since it indicates that in regions of low rainfall the application of sulphate sulphur is not likely to increase the organic sulphur in the plant.

The crude protein results are included in the last column to show the variation in this constituent compared with the organic sulphur. A cursory examination of these figures shows that there is no apparent correlation between them, which supports the conclusions arrived at in the discussion of Table I. This is apparent from a comparison of the organic sulphur and protein content in the early spring growth and in the May cutting. In spite of the fact that there is a decrease of about 6 per cent. in the crude protein in both the A and B plots in May, the organic sulphur is higher than in the spring growth. This can only be explained on the ground that either the protein constituents must vary considerably or that the sulphur must be present in some form other than protein sulphur.

It is also interesting to note that the herbage of plot B, on heavy clay soil, was distinctly higher in both total and organic sulphur compared with the figures obtained with the monthly growth on light sandy soil (Table I).

THE PROPORTION OF THE ORGANIC SULPHUR PRESENT AS CYSTINE.

Since the results of Aitken[5] with acid hydrolysis had failed to show the presence of cystine in pasture grass, it was decided, in the first instance, to carry out the hydrolysis by means of enzyme action. Although hydrolysis by enzyme action is slow and not so complete, it

was considered that any destruction of cystine that might occur with acid hydrolysis would be obviated.

In the first experiment, hydrolysis was carried out by means of pepsin. To 800 gm. of dried grass about 5 litres of distilled water were added and, after the reaction had been brought to pH 2 by the addition of HCl, 10 gm. of pepsin. The mixture was put in Winchester quart bottles and placed in an incubator at 37° C. for eight days, with frequent shaking. At the end of this period, the mixture was filtered and the residue was washed well with hot water. The filtrate was then evaporated *in vacuo* at a temperature of 50° C. to about 200 c.c. The residue, which was ink black in colour, was then transferred to a beaker and neutralised to litmus by the addition, with constant stirring, of NaOH, care being taken to prevent a rise in temperature. Acetic acid was added to bring the reaction to pH 5, and afterwards alcohol equal to twice the volume of the liquid. A copious brownish precipitate separated after standing for twenty-four hours. The precipitate was filtered and washed with water and alcohol.

The crude precipitate obtained was dissolved in 5 per cent. HCl and decolorised by boiling with charcoal (previously boiled with acid and washed). To recover any adsorbed cystine, the charcoal was again boiled with 5 per cent. HCl and washed with hot water. A yellowish filtrate was obtained, to which, on boiling, a hot 50 per cent. solution of sodium acetate was added until the reaction was at pH 4·9. On standing, a small white precipitate was obtained. A sulphur estimation carried out on this precipitate showed that it contained only a trace of sulphur. On testing the filtrate with Folin's reagent[4] a distinct blue colour was given, but no colour was obtained with the Sullivan reagent[2].

The negative result obtained in the above experiment might be due to the fact that pepsin only hydrolyses about 20 per cent. of the peptide linkages, whereas the Sullivan reagent gives a colour only with free cystine. To obtain a more complete hydrolysis, it was decided to use both pepsin and trypsin, with a longer period of incubation. To 200 gm. of grass, 2000 c.c. of distilled water were added and 5 gm. of pepsin, the mixture being brought to pH 2 as before. After incubation and frequent shaking for seven days, the mixture was allowed to cool and then brought to pH 8 by the careful addition of NaOH. Five gm. of trypsin were then added together with 20 c.c. of toluol. The digestion was then allowed to continue for a further seven days. The mixture was filtered and washed as before. The filtrate was evaporated *in vacuo* to about 200 cc. After neutralising to litmus and adding acetic acid to bring the

reaction to pH 5, two volumes of alcohol were added as above. A brownish precipitate separated out on standing and this was washed with water and alcohol and dried in the water oven at 100° C. The weight of precipitate obtained was 13·47 gm. and it contained 30 per cent. of ash.

In order to find whether any cystine was present, a total and inorganic 'sulphur determination was carried out on a portion of the precipitate. The total sulphur was found to be 2·00 per cent. and the inorganic sulphur was 1·71 per cent. The percentage of volatile or organic sulphur was, therefore, 0·29 per cent. Calculating on the 13·47 gm. of precipitate obtained, this is equivalent to 0·04 gm. sulphur or 0·15 gm. of cystine in 200 gm. of grass, assuming the volatile sulphur to have originated from cystine present.

Colorimetric estimations of cystine were also carried out on 1 gm. of the precipitate made up to 100 c.c. with $N/10$ HCl. For this purpose, 2 c.c. of the solution were taken and the cystine was estimated according to Rimington's [10] recent modification of Folin's method. The standard solution used for comparison contained 1 mg. of cystine per c.c. dissolved in $N/10$ HCl. By this method 1 gm. of the precipitate was found to contain 32·5 mg. of cystine, which, calculated on the 13·47 gm. of precipitate obtained from 200 gm. of grass, is equivalent to 0·22 per cent. of cystine on the dry matter.

The cystine was also estimated by the Sullivan method (1929). A precipitate, however, developed and only a very slight red colour was obtained showing hardly a trace of cystine to be present.

The remainder of the precipitate was decolorised with charcoal and reprecipitated with sodium acetate and alcohol. A small white precipitate was obtained but microscopical examination failed to show any crystals of cystine to be present.

To test the applicability of the above methods, 200 gm. of grass were again hydrolysed with pepsin and trypsin under the requisite conditions, the hydrolysis being carried out in duplicate. After evaporating *in vacuo*, 1 gm. of pure cystine was added to one of the digest solutions before precipitation with sodium acetate and alcohol. The weight of dry precipitate obtained from 200 gm. of grass without cystine addition was 8·95 gm., and with the addition of cystine 14·80 gm. One gm. of each precipitate was made up to 100 c.c. with $N/10$ HCl as before. Folin's modified method gave 0·22 gm. of cystine per 100 gm. grass with the former and 1·575 gm. of cystine (on 14·80 gm. precipitate) or 0·29 per cent. cystine from the grass with the latter.

Five c.c. of each solution were tested with the Sullivan reagent. The solution containing added cystine gave a strong positive test, but a precipitate again developed so that it was impossible to estimate the amount present. The solution without cystine addition gave only a faint red colour.

In view of the extremely low figures for cystine obtained with pasture grass, using enzyme hydrolysis, it was decided to carry out the hydrolysis by means of 25 per cent. (by vol.) H_2SO_4. 100 gm. of grass were hydrolysed with 600 c.c. of 25 per cent. H_2SO_4 for twenty-four hours. The solution was filtered and the black residue was washed well with hot water. The filtrate was then diluted until it contained 5 per cent. H_2SO_4. The cystine was precipitated by means of mercuric sulphate in 7 per cent. H_2SO_4 according to Hopkin's method: After standing twenty-four hours the precipitate was filtered and washed well with 5 per cent. H_2SO_4 to remove tyrosine and afterwards with water. The precipitate was decomposed with H_2S and the sulphuric acid was removed with baryta. The solution was then evaporated *in vacuo* to a low volume. The residue was transferred to a dish and a drop of ammonia was added to make it slightly alkaline and a trace of iron to oxidise any cysteine present. It was then evaporated slowly on the water bath until crystallisation occurred. Alcohol was then added and the precipitate was allowed to stand. On examining the crystals under a microscope a few hexagonal platelets, typical of cystine, were observed. The amount present was small and contaminated with impurities, but, nevertheless, it suggests that a trace of cystine is present in grass.

In a further attempt to estimate the cystine present, 50 gm. of week-old pasture grass were first extracted with ether to remove most of the colouring matter, and then hydrolysed with both pepsin and trypsin under the requisite conditions. After filtering, 200 c.c. portions of the filtrate were taken and both the total and inorganic sulphate were estimated. The total sulphur in the 2000 c.c. of filtrate from 50 gm. of grass was 0·44 gm. and the inorganic sulphur was 0·24 gm. The organic sulphur present in the solution was therefore 0·20 gm. This is equivalent to 1·8 mg. of cystine per 5 c.c. on the assumption that the organic sulphur is present as cystine. Five c.c. of the filtrate were, therefore, taken for cystine estimation according to Rimington's modification of Folin's method. A deep blue colour was obtained, and although a slight difference in tint made comparison between it and the standard solution not very satisfactory, a cystine content of over 1 per cent. was indicated in the grass. The Sullivan reagent, again, gave only a slight reddish colour,

while the nitro-prusside test was negative. In view of the latter evidence one is forced to the conclusion that some interfering substance is present which gives a blue colour with Folin's reagent but is not precipitated by sodium acetate and alcohol.

Briefly summarised, therefore, attempts to isolate cystine from pasture grass have shown only a trace of the amino acid to be present. The Folin method applied to the precipitates gave 0·22, 0·22 and 0·29 per cent. of cystine in grass, while the Sullivan method indicated only a trace of cystine present.

THE ALKALI-LABILE SULPHUR OF PASTURE GRASS.

It is well known that the isolation of cystine from protein hydrolysates, especially when present in small quantities, is a matter of considerable difficulty. Further proof was therefore sought to confirm the above conclusions as to the cystine content of young grass. A useful indication of the existence and amount of cystine in a protein can be obtained by boiling with 30 per cent. NaOH and estimating the sulphide sulphur formed as lead sulphide. The method is based on the fact that free cystine yields two-thirds of its sulphur as sulphide on heating with alkali. It is assumed that the cystine present in the protein molecule behaves in a similar manner.

That protein sulphur other than that present as cystine gives rise to sulphide sulphur, however, is shown by the following comparisons given in Table III.

Table III. *Giving a comparison of the cystine content of proteins as calculated from the sulphide sulphur figures (on the basis of cystine yielding two-thirds of its sulphur as sulphide) with the results obtained by colorimetric methods.*

Protein	Total sulphur (11) (%)	Sulphide sulphur (11) (%)	Sulphide sulphur calculated as cystine (%)	Cystine determination colorimetrically (12) (%)
Casein	0·800	0·100	0·56	0·26–0·29
Vicilin	0·200	0·092	0·52	0·31–0·34
Egg albumin	1·616	0·489	2·75	1·21–1·39
Gliadin	1·031	0·620	3·50	2·00–2·70
Phaseolin	0·312	0·071	0·40	0·00–0·34

The figures in the third column of Table III are invariably higher than those obtained for the cystine content using colorimetric methods. Naturally, the latter are again higher than the amount of crystalline cystine actually isolated from these sources. It seems, on the other

hand, that the cystine calculated from the sulphide sulphur results is "roughly proportional" to the true cystine content and, therefore, can serve as a useful guide to the amount of the amino acid present.

To determine the sulphide sulphur liberated on heating with 30 per cent. alkali, the following modification was introduced. 25 gm. of dried grass (10 per cent. moisture content) containing, therefore, about 5 gm. of protein were boiled gently on a sand bath under a reflux condenser with 100 c.c. of 30 per cent. NaOH for about five hours. After cooling, the mixture was transferred to a medium-sized suction flask. The flask was closed with a cork through which passed a dropping funnel and also a bent glass tube passing to the bottom of the flask. The latter was connected to a bottle containing lead acetate so that any air entering the flask had to pass first through this bottle. The side tube of the flask was also connected to two absorption bottles containing lead acetate solution, and these were again connected to a water pump so that a steady current of air could be drawn through the whole apparatus. The mixture in the flask was then acidified with HCl by means of the dropping funnel. The addition of excess acid was immediately indicated by the formation of black lead sulphide in the first absorption bottle. That absorption was complete is indicated by the fact that no blackening occurred in the second bottle. To ensure that all the H_2S had been brought over, the second absorption bottle was connected to the flask and a current of air was drawn through for another hour. The contents of the absorption bottles were then transferred to a beaker and acidified with acetic acid to decompose any carbonate formed during aeration. The solution was filtered through a small filter paper and washed well with cold water to remove all the lead acetate. After drying, the filter paper was transferred to a weighed crucible and heated gently to convert the sulphide into sulphate. Finally a few drops of conc. H_2SO_4 were added so as to ensure that all the lead was present as sulphate. The crucible was again heated, at first gently and afterwards strongly and then cooled and weighed. The results obtained were as follows.

(1) *Weekly cut grass*. Percentage organic sulphur present was 0·36. Weight of $PbSO_4$ from 25 gm. grass (10 per cent. moisture content) = 0·0471 gm. Assuming cystine to liberate two-thirds of its sulphur as sulphide under these conditions, the cystine content of the grass is 0·12 per cent. of the dry matter.

(2) *Two weeks old grass*. Organic sulphur = 0·47 per cent. Weight of $PbSO_4$ obtained = 0·0258. This is equivalent to 0·07 per cent. cystine on the dry matter basis.

(3) *Three weeks old grass.* Weight of $PbSO_4 = 0.0327$ gm. equivalent to 0.08 per cent. cystine on the dry matter.

It is evident that these results fully confirm the conclusions already given above as to the cystine content of young grass. They indicate a cystine content of less than 0.1 per cent. on the dry matter.

A criticism that might be brought forward, however, is that the lability of the sulphur towards alkali had been modified by the drying process. To test this point, two samples of fresh grass were passed through a mincing machine and the pulp was divided into three parts. The first portion of 50 gm. was placed directly in the flask and acidified with HCl, in the manner already described, so as to find whether any sulphide sulphur was present in the grass. The results obtained were negative. The second portion of 100 gm. was boiled with alkali, while the third portion was first dried at 100° C. and then heated with alkali. The results obtained were as follows.

	Sulphide sulphur on wet grass (dry matter basis) (%)	Sulphide sulphur on same grass dried at 100° C. (dry matter basis) (%)
(1)	0.023	0.017
(2)	0.048	0.021

It will be observed that a reduction occurred in the sulphide sulphur on drying at 100° C. Even on the wet grass, however, the maximum sulphide sulphur corresponds only to 0.18 per cent. of cystine. All the evidence points to the fact that young pasture grass is deficient in cystine.

SUMMARY AND CONCLUSIONS.

1. Organic sulphur determinations have been carried out on pasture grass at different stages of maturity. A decrease of about 39 per cent. was found in this constituent when the grass was cut for hay compared with the weekly and fortnightly stages of growth.

2. The application of heavy dressings of inorganic sulphates to a good heavy clay permanent pasture produced an average increase of 8 per cent. in the organic sulphur content.

3. No correlation was found between the organic sulphur and protein content of pasture grass, which suggests that the organic sulphur is present in part, at least, as non-protein sulphur.

4. Attempts to isolate cystine from pasture grass indicate only a trace of the amino acid to be present. The Folin colorimetric method, when applied to precipitates, gave a cystine content of from 0.22 to

0·29 per cent. of the dry matter, but when applied directly to an enzyme hydrolysate of young grass it indicated a cystine content of over 1 per cent. The Sullivan colorimetric method showed that only a trace of cystine was present, although the addition of cystine to the hydrolysate proved the method to be applicable.

5. Further proof that pasture grass is deficient in cystine was obtained by determining the sulphide sulphur liberated on heating with 30 per cent. alkali. The results show a cystine content of 0·07 to 0·18 per cent. of the dry matter. Since other sulphur compounds seem to give rise to sulphide sulphur under these conditions, the cystine content of the grass must be less than 0·1 per cent. of the dry matter.

In view of this evidence one is forced to the conclusion that some precursor of cystine is present in grass which can be converted into cystine in the animal body. Further investigations on the nature of the organic sulphur will be carried out in the near future.

In conclusion I wish to express my thanks to Dr H. E. Woodman for the interest he has taken in this work.

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THE INFLUENCE OF THE PLANT UPON SEASONAL CHANGES IN SOIL ACIDITY.

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(With Four Text-figures.)

THE pH value of a soil is not constant, and fluctuations which have been reported [3, 9, 15] are probably related to the seasonal variations found in the concentration of electrolytes in the soil [4, 5, 7, 14]. For example, the pH value usually decreases during summer or during drought, at which times the concentration of electrolytes increases, especially under fallow conditions. It is to be expected, therefore, that the growing plant will affect the acidity of the soil, and this paper is a report of results which demonstrate that point for the potato (*Solanum tuberosum*) and a variety of soils.

EXPERIMENTAL.

The work is considered under the three headings, incubation, pot and field experiments.

Determination of p*H*. The soils were usually examined soon after sampling, by means of the quinhydrone electrode, the procedure being essentially the same as that recently recommended [12]. A number of "10 second values" [12] showed that there was no drift due to the presence of manganese dioxide in any of the soils under investigation.

Incubation experiments.

Methods. The following soils were passed through a 2 mm. sieve for these experiments.

Soil B. The mechanical analysis of this soil by the International Pipette Method [2] gave the following percentage composition: coarse sand 20, fine sand 26, silt 24, clay 25, air dry moisture 3·7, loss on ignition 7·4.

Soil P. The sample was taken in March from an uncultivated peaty layer 9–10 in. deep overlying well weathered basic andesite. The loss on ignition of the oven-dry material was 48 per cent.

Soil W. A light sandy soil taken from a mound of glacial sand, which

has not been cultivated for at least 60 years. The loss on ignition, due largely to partially decayed organic matter, was 9 per cent., whilst coarse sand amounted to 50 per cent., and fine sand to 20 per cent.
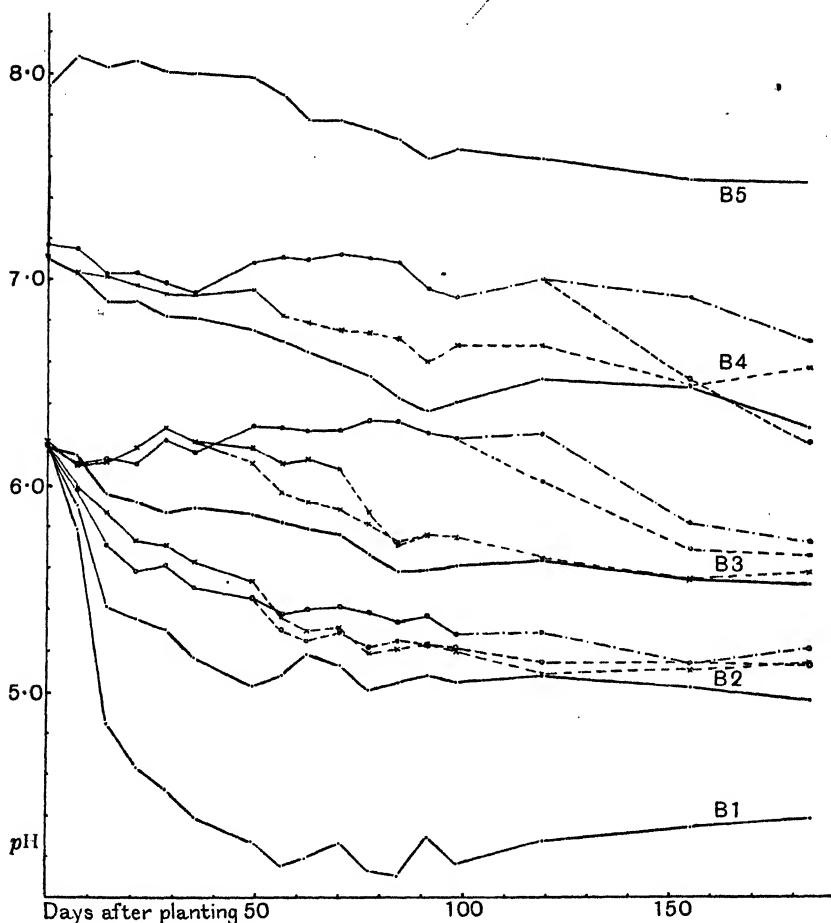

Fig. 1. —·— Fallow —o— Golden Wonder —×— Epicure.

Soil G. A sandy soil, from an uncultivated raised beach close to the south shore of the Firth of Forth, having 68 per cent. coarse sand, 10 per cent. fine sand, 8·5 per cent. loss on ignition.

In addition, samples of B, which had an initial pH value of about 6·2, were treated with 0·35 and 0·12 per cent. calcium hydroxide and with 0·024 and 0·12 per cent. sulphur to obtain a range of pH values

from about 8 to 4. Similarly, samples of P, having an initial pH value of 3·6, were treated with 1·0 and 2·0 per cent. calcium hydroxide to obtain pH values of about 5 and 6. Soils W and G, of which the original pH values were about 4·5 and 6·7 respectively, were examined only in the untreated condition. The treatment was calculated in each case for oven-dried soil.

The soils, which had not reached an air-dry condition, were brought to moisture contents suitable for plant growth, and 500 gm. portions were placed in glass dishes. Small pieces of potato tubers of the varieties Epicure and Golden Wonder were planted in each sample of soil, excepting B 1 and B 5. The temperature was maintained between 18° and 20° C. throughout the experiments. The dishes were watered daily and small samples of soil were removed at regular intervals for pH determinations. The values obtained for B are shown graphically in Fig. 1. Each curve represents the average of duplicates until certain of the plants died. For example, in Fig. 1 the curve for Epicure in sample B 3 represents the average value for the two plants up to 35 days; about that time the plant in one dish died and the broken line shows the subsequent value for the soil in that dish: at 70 days the plant in the second dish died and there was a rapid decrease in pH value until at 90 days the soils in the two dishes were again giving practically the same pH values. Where the solid lines give place to alternate dash and dot, as, for example, with most of the Golden Wonder plants at 90 days, it indicates that the plants were "nipped" to stop growth. Those plants were very vigorous, however, and in some cases still survived when the experiment was concluded, although growth had practically stopped. The curves for soils P, W and G were essentially the same as those for B 3 and B 4.

Results. In all the unplanted soils there is a fairly steady decrease in pH value during the first 70–100 days and thereafter the changes are small and irregular. In the case of the sulphur treated samples, B 1 and B 2, the results are very similar to those reported by other workers (1, 6, 8). In the soils supporting plants there was usually a slight fall in pH value until the shoots were established, and then a rise during the rapid stages of growth (except in the case of B 2) to a point higher than the initial value. The pH value then decreased, fairly rapidly after the death of a plant, until it approached the value for the unplanted soil. That B 2 should prove an exception was apparently due to the fact that the normal influence of the growing plant in preventing an increase in acidity was more than counteracted by the oxidation of the sulphur in

increasing the acidity. Evidence in support of that explanation is supplied first of all by the fact that the sulphur produced a greater increase in acidity in the fallow than in the planted soil, and secondly by the results (Fig. 2) obtained in an experiment with a portion of soil B which had been treated with an amount of dilute sulphuric acid equivalent to an addition of 0·02 per cent. sulphur.

Fig. 2.   — · — Fallow   —o— Golden Wonder   — × — Epicure.

The curves obtained are quite similar to those for B 2 after 70–80 days when the oxidation of sulphur was apparently complete. The decrease in acidity due to the rapidly growing plant and the subsequent return, on "nipping," to that of the unplanted soil are very well marked.

In order to make sure that the differences in pH values between unplanted and planted soils were not due to differences in moisture content effected by the growing plant, a number of soils were maintained at definite moisture contents, by daily watering, for a period of 30 days. Measurements of pH were made at intervals and the final values are reported in Table I. The results show that different amounts of moisture in the soil, over a range of 10 per cent. in the neighbourhood of the optimum moisture content, have very little effect upon the changes in acidity.

Table I. pH values after 30 days at constant moisture content.

Soil	B + 0·025 % S(H$_2$SO$_4$)			B			B + 0·125 % Ca(OH)$_2$		
% moisture	15	20	25	15	20	25	15	20	25
pH	5·23	5·25	5·28	5·89	5·85	5·84	6·86	6·87	6·90

Soil	W			G		
% moisture	10	15	20	10	15	20
pH	4·42	4·37	4·38	6·24	6·28	6·31

It has been shown (13) that a large increase in the quantity of electrolytes in a soil may occur if it is maintained under conditions suitable for

biological activity. Such an increase modified by the absorption of salts by the plant would account for the results obtained for the fallow soils and the early observations for the planted soils. A return of electrolytes from the decaying plant to the soil is a possible explanation for the final observations for the planted soils, but this point requires further investigation.

Pot experiments.

Methods. A large sample of soil B, taken from the field in March, was employed in these experiments. While still moist it was broken up, passed through a ¼ in. riddle and divided into three portions. One
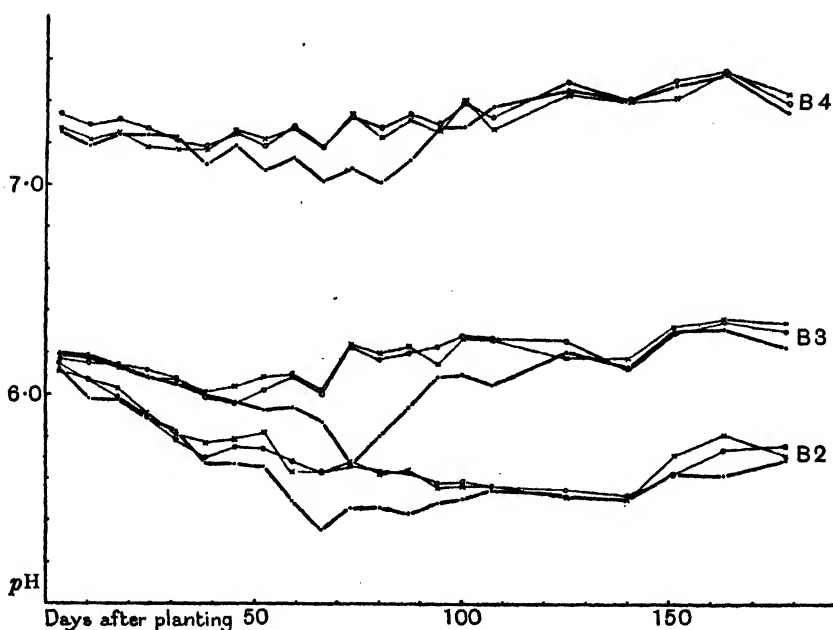
Fig. 3. — • — Fallow — o — Golden Wonder — × — Epicure.

portion (B 2) received 0·024 per cent. sulphur, the second (B 3) was untreated, and the third (B 4) received 0·12 per cent. calcium hydroxide. The additions were calculated in terms of oven-dry soil. Ten pots (12 in. diameter) were filled from each portion and potatoes were planted on April 14. Four pots of B 2 were planted with the variety Epicure, four with Golden Wonder, and two pots were kept fallow. The same was done for the series B 3 and B 4. Samples of soil were taken at weekly intervals until August, and then less frequently until October, by means

of a small auger which reached to the bottom of the pots. During the ˌ warm, dry weather of May and June the pots had to be watered fre-ˌ quently, and that probably accounts for the fluctuations and total changes in acidity being small compared with those found in the field experiments described later. The results are shown graphically in Fig. 3.

ˈResults. Considering the fallow pots first, it will be observed that the pH value of each soil decreased regularly to a minimum whiˌch, e̊xcept for B 4, existed only for a brief period. Those minima were reached after practically the same length of time as in the incubation experiments, but the total changes in acidity were not so great in this case. The pH value then increased, rapidly at first and then more slowly, so that by October 9 it had returned to the origiˌnal value except in the case of B 2. During the first 30–40 days the soils of the planted pots did not differ ˙materiäÌly as regards acidity from those of the fallow pots. The Epicure plants appeared above the surface of the soil in the period 30–40 days, and the Golden Wonder in the period 40–50 days after planting. (The curves for the late variety, Golden Wonder, will be observed to lag some-- what behind those for the early maturing variety, Epicure.) From the time when the shoots appeared until about 60 or 70 days later when the haulms were withering, the pH values of the planted soils were definitely higher than those of the corresponding fallow soils. In all three series there may be observed a slight increase in pH value when the shoots appeared, which is interesting in view of the similar observa- tion in the case of the incubation experiments. The difference in acidity between planted and fallow soils increased until the plants were flowering, which corresponded approximately with the period of minimum pH values for the fallow soils: the difference then became less until even- tually the curves for planted and fallow soils were practically the same.

Field experiments.

(a) Plot experiment. A small area at the College Experimental Farm, Boghall (soil B), was divided into five plots which received the following treatments per acre: plots B 1 and B 2, 1200 and 400 lb. sulphur respec- tively, B 3 no treatment, B 4 and B 5, 1400 and 4200 lb. calcium hydroxide respectively. The calcium hydroxide was applied in November, 1929, the flowers of sulphur at the beginning of April, 1930. The dressings were approximately the same as those in the incubation and pot experiments already described. At the beginning of May, part of each plot was planted with potatoes. At the end of June, four plants were marked in each plot and thereafter soil samples were always taken from the roots ˌ

of each marked plant and from the middle of each fallow area. The first plants appeared at 25 days, the flowers and small tubers had formed at 70 days, the crop was harvested about 150 days after planting.

Results. The fluctuations and total changes in acidity were very great, but the remarkable parallelism amongst the results for all plots suggested the probability of a common factor and some justification for simplifying the presentation of the data by averaging the results for the five fallow and those for the five planted areas. The "average" curves are presented in Fig. 4.
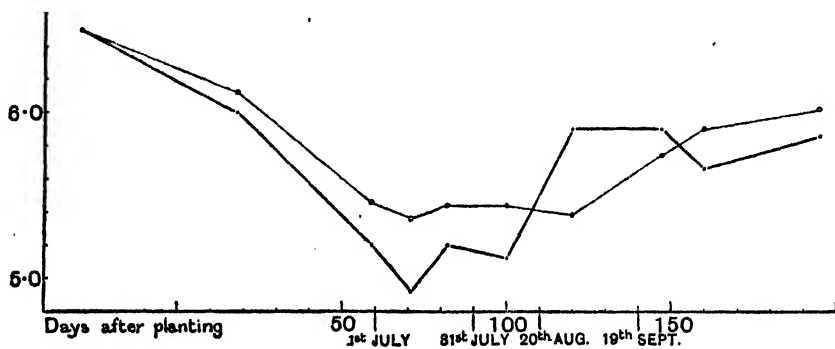

Fig. 4. —·— Fallow —o— Planted.

As in the case of the pot experiments, the curves gradually diverge until about 70 days after planting, at which point the pH value of the fallow soils had reached a minimum. The pH value of the planted soils remained practically constant during the next 50 days and then gradually increased. The value for the fallow soils, on the other hand, fluctuated considerably, but at 160–190 days had approached close to that for the planted soils.

(b) *Field experiments.* Eight soils, within a small area of about 6 miles radius but showing considerable variation in their chief physical and chemical properties, were studied under field conditions in 1929 and 1930. The manurial treatment was similar in all cases, and soil samples were taken as described under the preceding section. The first samples were not taken, however, until the end of June when the crop was fairly well advanced, and a consideration of Fig. 4 shows that a large increase in acidity had taken place by that date at a comparatively "late" locality. Consequently, the first results recorded for the field soils were probably not far removed from the minimum pH values for the season. The fluctuations recorded in the later part of the growing

season when, generally speaking, the reaction of the soil is tending to become less acid, seemed to be related to the changes in temperature and moisture conditions rather than to stage of plant growth.

Results. A comparison of the data for the two seasons is limited to a few outstanding features.

In 1929. (*a*) From July 29 to August 18—moderate temperature and high rainfall—there was an increase or little change in *p*H values. (*b*) From August 18 to September 20—high temperatures and moderate rainfall—there was a decrease in *p*H value for the more acid soils and an increase in *p*H value for the less acid soils. (*c*) The final readings, made at the beginning of October, after a spell of cool, wet weather, showed a general increase in *p*H values.

In 1930. (*a*) From July 12 to 23—normal temperature and high rainfall—there was an increase in *p*H values, with two exceptions, for both planted and fallow soils. The plants were not so far advanced as in the similar period (*a*) in 1929, which probably accounts for the more definite change. (*b*) From August 10 to September 2—high temperatures and high rainfall—the planted soils showed somewhat similar tendencies in acidity changes as in (*b*) 1929, whereas all the fallow soils showed a very marked increase in *p*H. In every case the fallow soils became less acid than the planted soils in this period (cp. Fig. 4). (*c*) The final readings, made in October, after a period of cool, wet weather, showed an increase in the *p*H values of planted and fallow soils.

The maximum change for the planted soils varied from 0·1 to 1·4 *p*H units and, generally speaking, the observations on the amount and irregularity of the variations for different soils agreed with reports by other workers (3, 9, 10, 11). None of the soils considered (except plots B 4 and B 5) had received lime for a number of years; the crop was the same in all cases and the manuring was similar: for each year the variation in climate can be regarded as negligible over such a small area: consequently the irregularity of the acidity changes may be ascribed to soil characteristics. In this respect it is noteworthy that at one locality the average *p*H value of the samples increased from 6·2 to 6·9 in 1929 and from 6·4 to 7·0 in 1930.

CONCLUSIONS.

In the early part of the season, when an accumulation of salts takes place (4, 5, 14) on account of the general rise in temperature, the acidity of the soil increases irrespective of climatic factors. Where plants are growing, that accumulation is not so great and the increase in acidity is

not so marked. Towards the end of the growing season, climatic factors, and particularly rainfall, appear to be responsible for irregular fluctuations, but there is a general tendency for the acidity to decrease. Finally, the pH value, like the concentration of salts (7), becomes approximately the same for both planted and fallow soils and not far removed from that at the beginning of the season.

It seems, therefore, that variations in soil acidity are definitely connected with changes in the quantities of electrolytes present, and that the effect of the plant is due, at least in part, to absorption of salts. It is possible, however, that bicarbonates formed as a result of plant growth also exert some influence.

SUMMARY.

1. A large number of observations on different soils in incubation, pot and field experiments have been made to determine the effect of the growing plant upon seasonal changes in soil acidity.

2. In every case it has been shown that the acidity of the uncropped soil increases to a maximum during the growing season and that the change may amount to more than one pH unit.

3. The plant reduces the change in acidity to a marked extent and at the height of growth there is a considerable difference between the acidity of the cropped and uncropped soil.

4. At the end of the growing season that difference has practically disappeared and the acidity of the soil approaches the value found at the beginning of the season.

5. Irregular fluctuations are related to climatic factors.

6. Attention has been directed to the analogous variations found in the concentration of electrolytes in the soil.

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A COMPARISON OF TWO AGAR MEDIA FOR COUNTING SOIL MICRO-ORGANISMS.

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IT was found in the study carried out by Fisher, Thornton and Mackenzie[2] on the accuracy of the plate method for estimating bacterial numbers, that at least one medium, viz. the mannite-asparagine agar devised by Thornton[5], will, even with the mixed population of the soil, give results which are in fair agreement with theoretical requirements, namely that the numbers of bacterial colonies on the plates shall depend solely upon the numbers of bacterial cells in the inoculum which are able to develop on the said medium. When this is the case, values of the index of dispersion χ^2, calculated by the formula

$$\chi^2 = \frac{S\,(x - \bar{x})^2}{\bar{x}}$$

(where x is the number of colonies on each plate, $\bar{x}$ the mean), are distributed according to a definite law (Fisher[1]), and the average of these values is equal to the number of degrees of freedom, *i.e.* one less than the number of parallel plates.

In their comments upon a paper by Smith and Worden[4], in which the numbers of bacteria in a soil were determined on three agar media, Thornton and Fisher[6] point out that only one of these media—the mannite-asparagine agar—has been tested for its χ^2 distribution. It would therefore be desirable to extend this test to other agar media. The author found an opportunity of doing this when carrying out a number of bacterial counts in soils during some experiments on the decomposition of farmyard manure and dead microbial protoplasm. The material also provided an opportunity of testing the χ^2 distribution for counts of actinomycetes colonies. The soils used were:

1. Heavy clay soil, rich in organic matter and of neutral reaction, from Rothamsted grass plots; with and without addition of farmyard manure and straw.

2. Light sand soil, poor in organic matter and of faintly acid reaction,

from Woburn Experimental Farm; with and without addition of lime, farmyard manure, and straw.

3. Heavy clay soil, poor in organic matter and of faintly acid reaction, from unfertilised plot on Hoos Field, Rothamsted.

4. Another sample from the same field.

5. Heavy loam, rich in organic matter, from a plot of garden soil outside the laboratories of Rothamsted Experimental Station.

The soils were kept in a moist condition in the laboratory for periods up to 500 days, some at room temperature and some at 25° C. The two agar media, on which counts were carried out, were:

1. Mannite-asparagine agar (Thornton (5)).

2. A modification of the dextrose-casein agar mentioned by Waksman (7): dextrose, 2·0 gm.; casein dissolved in 0·1 N NaOH, 0·2 gm.; K_2HPO_4, 0·5 gm.; $MgSO_4$, 0·2 gm.; $FeCl_3$, trace; agar, 15·0 gm.; distilled water, 1000 c.c. of pH 6·4–6·6.

Counts on these two media were made in eighty instances, mostly from soils 1 and 5. Plates were poured on both media from the same soil suspension, the concentration of which ranged from 1:100,000 to 1:2,000,000 according to the numbers of micro-organisms in the soil. The plates were incubated for the same period (10 days) in the same incubator (20° C.). The number of parallel plates ranged from 4 to 10, and was in most cases 5 or 6. Plates obviously spoiled by fungi or fluorescent bacteria were discarded. The average numbers of bacterial colonies per plate ranged from 44 to 460 on mannite agar, and from 54 to 796 on dextrose agar. The corresponding limits for the numbers of actinomycetes colonies were 2 and 110 on mannite agar, 6 and 97 on dextrose agar. The number of bacterial colonies in 74 cases out of 80 was higher on dextrose than on mannite agar, in several cases 2 to 4 times as high. The significance of these differences was tested by calculating the following statistics (Fisher (1)):

$$\text{Standard deviation} \quad s = \sqrt{\frac{S\,(x_1 - \bar{x}_1)^2 + S\,(x_2 - \bar{x}_2)^2}{n_1 + n_2}},$$

and
$$t = \frac{\bar{x}_1 - \bar{x}_2}{s} \sqrt{\frac{(n_1 + 1)\,(n_2 + 1)}{n_1 + n_2 + 2}},$$

where x_1 and x_2 = numbers of colonies on individual parallel plates of the two media, $\bar{x}_1$ and $\bar{x}_2$ = means of numbers of colonies, n_1 and n_2 = degrees of freedom (number of parallel plates − 1).

From Fisher's table of t the probability, P, of the difference being

accidental was found. If P was less than 0·05 the difference was considered significant.

Table I. *Comparison between numbers of colonies of bacteria and actino-mycetes on mannite-asparagine agar* (MA) *and dextrose-casein agar* (CA).

Ratio Colonies CA / Colonies MA	Bacteria		Actinomycetes	
	Occurrences	Cases of significant difference	Occurrences	Cases of significant difference
0·60–0·79	0	0	3	1
0·80–0·99	6	1	6	0
1·00–1·19	20	8	21	3
1·20–1·39	20	19	18	13
1·40–1·59	13	13	11	11
1·60–1·79	8	8	6	6
1·80–1·99	4	4	6	6
2·00–2·19	3	3	2	2
2·20–2·39	3	3	1	1
2·40–2·59	1	1	0	0
2·60–2·79	1	1	2	2
2·80–2·99	0	0	0	0
3·00–3·19	0	0	2	2
3·20–3·59	0	0	0	0
3·60–3·79	0	0	1	1
3·80–4·00	1	1	0	0
Above 4·00	0	0	1	1
Total	80	62	80	49
Lowest ratio	0·85		0·74	
Highest ratio	3·94		4·50	

Table I shows that only in one case was the number of bacteria significantly higher on mannite agar than on dextrose agar, whereas the reverse occurred in 61 cases. The number of actinomycetes, too, was significantly higher on mannite agar in only one case, but significantly higher on dextrose agar in 48 cases.

The values of χ^2 for bacteria and actinomycetes obtained with mannite-asparagine agar are shown in Table II. Since we were dealing with several sets with varying numbers of parallel plates, use was made of the method shown by Fisher[1] for testing the reliability of the counts under those conditions. The totals of observed χ^2 and degrees of freedom (n) are summed up; if the difference $\sqrt{2\chi^2} - \sqrt{2n-1}$ exceeds $+ 2$ or is less than $- 2$, the value of χ^2 is not in agreement with expectation, *i.e.* the variability is either excessively high or abnormally low.

The χ^2 for bacteria was not as a whole outside the range of expectation (as also found by Fisher, Thornton and Mackenzie[2]), although there seems to have been a tendency to excess in the few 4-plate sets.

The χ^2 for the actinomycetes, on the other hand, showed a definitely subnormal variation, which, according to Fisher, Thornton and Mackenzie [2], is usually due to some defect in the medium.

Table II. *Values of χ^2 in counts of bacteria and actinomycetes on mannite-asparagine agar.*

Plates in set $(n+1)$	Numbers of sets (S)	$S(n)$	Totals of χ^2	
			Bacteria	Actinomycetes
4	3	9	24·33	7·71
5	20	80	79·67	66·76
6	43	215	237·55	167·43
7	8	48	60·51	26·88
8	4	28	33·74	26·27
9	2	16	8·06	5·04
Total	80	396	443·86	300·09
Difference $\sqrt{2\chi^2} - \sqrt{2n-1}$			+1·67	−3·62

A much larger number of counts was carried out on the dextrose-casein agar in order to get sufficient data for a study of the distribution of χ^2 on this medium, which has not previously been tested in this respect. The counts included in all:

$$2 \text{ sets with 3 parallel plates.}$$

33	,,	4	,,
270	,,	5	,,
38	,,	6	,,
11	,,	7	,,
2	,,	8	,,
1	,,	9	,,

The distribution of χ^2 in the 270 5-plate sets is shown in Table III. (Values of χ^2 and expected frequencies taken from Fisher [1], *Table III.*)

The χ^2 for the bacterial counts showed upon the whole a good agreement with expectation, with some deficiency in the lower and some excess in the higher classes of variation. This medium thus seemed to be much like the mannite-asparagine agar in this respect, since it had only a slight tendency to give excessive variation; this is also the case with mannite-asparagine agar, as shown by Fisher, Thornton and Mackenzie, and as seen from Table VI. The χ^2 from counts of actinomycetes agreed almost perfectly with expectation in the lower and the very high classes. In the classes of χ^2 from 4·9 to 9·5 there was a deficiency, and in the classes near the mean ($\chi^2 = 4$, equal to the number of degrees of freedom) there was a corresponding excess.

The counts in the rest of the sets gave χ^2 values of a similar character,

as shown in Table IV. Here again the observed values of χ^2 for both bacteria and actinomycetes are, as a whole, in good agreement with expectation.

Table III. *Distribution of χ^2 in counts of bacteria and actinomycetes on dextrose-casein agar (5-plate set).*

χ^2	Expected	Observed	
		Bacteria	Actinomycetes
0			
0·297	3	0	1
0·429	3	2	3
0·711	7	6	11
1·064	14	8	14
1·649	27	19	28
2·195	27	17	24
3·357	54	57	62
4·878	54	64	69
5·989	27	21	20
7·779	27	29	16
9·488	14	20	9
11·668	7	16	7
13·279	3	8	3
	3	3	2
Total	270	270	269

Table IV. *Values of χ^2 in counts of bacteria and actinomycetes on dextrose-casein agar.*

Plates in set $(n+1)$	Number of sets (S)	$S(n)$	Totals of χ^2	
			Bacteria	Actinomycetes
3	2	4	4·91	2·37
4	33	99	148·90	137·15
6	38	190	192·39	142·76
7	11	66	73·45	50·37
8	2	14	7·19	13·48
9	1	8	3·12	4·05
Total	87	381	429·96	350·18
Difference $\sqrt{2\chi^2} - \sqrt{2n-1}$			$+1\cdot73$	$-1\cdot12$

The data treated here have been obtained from several soils of different character, and moreover the additions of various organic materials to the soils have given rise to development of widely different microfloras in the different soils. In Tables III and IV, as well as in Table II, all the data have been combined, with the possibility that some of the series may have shown excessive, others subnormal variation, and that these abnormalities may have compensated each other in the final result. To check this, the figures in Table V have been calculated. For 33 different series of counts on casein agar (6 without and 27 with addition of organic material) the values of χ^2 and n have been summed up; when $S(n)$ exceeded 30, the difference $\sqrt{2\chi^2} - \sqrt{2n-1}$

has been calculated, and when $S(n)$ was less than 30, the value of P, taken from Fisher's Table III, has been recorded. When this value exceeded 0·95 or was less than 0·05, the variation was considered abnormal.

Table V. *Values of χ^2 in different soils. Counts on dextrose-casein agar.*

Soil no.	Addition		Number of sets (S)	$S(n)$	Total χ^2	Difference $\sqrt{2\chi^2} - \sqrt{2n-1}$
		Soils without organic matter.				
2	Nothing	Bact.	13	51	55·00	0·44
		Act.	13	51	51·86	0·13
2	CaCO₃	Bact.	14	52	51·65	−0·01
		Act.	14	52	66·55	1·39
3	Nothing	Bact.	11	43	40·31	−0·24
		Act.	11	43	31·80	−1·24
4	Nothing	Bact.	8	32	39·19	0·92
		Act.	8	32	27·92	−0·46
1	Nothing	Bact.	17	89	102·20	1·00
		Act.	17	89	79·93	−0·65
5	Nothing	Bact.	16	70	92·58	1·78
		Act.	16	70	56·82	−1·13
	Total	Bact.	—	337	381·13	1·77
		Act.	—	337	314·19	−0·84
		Soils with organic matter.				
2	Farmyard manure	Bact.	13	51	55·02	0·44
		Act.	13	51	63·64	1·22
2	Do. + (NH₄)₂SO₄	Bact.	13	50	47·73	−0·18
		Act.	13	50	59·54	−0·95
2	Do. + Do. + oat straw	Bact.	12	46	45·79	−0·03
		Act.	12	46	43·18	−0·26
2	CaCO₃ + manure	Bact.	12	48	*84·52*	*3·25*
		Act.	12	48	38·76	−0·95
2	Do. + Do. + (NH₄)₂SO₄	Bact.	13	52	47·83	−0·37
		Act.	13	52	43·80	−0·79
2	Do. + Do. + Do. + oat straw	Bact.	14	54	64·86	1·05
		Act.	14	54	53·51	−0·02
1	Farmyard manure	Bact.	17	78	86·64	0·71
		Act.	17	78	*51·86*	*−2·27*
1	Do. + oat straw	Bact.	17	77	99·74	1·75
		Act.	17	77	*51·11*	*−2·26*
3	Farmyard manure	Bact.	11	47	38·08	−0·91
		Act.	11	47	38·95	−0·81
3	"Edelmist"	Bact.	11	44	48·39	0·51
		Act.	11	44	33·18	−1·18
4	Farmyard manure	Bact.	8	32	33·83	0·30
		Act.	8	32	27·37	−1·53
4	Do. dried	Bact.	8	32	*63·10*	*3·29*
		Act.	8	32	32·88	0·17
4	Synthetic farmyard manure	Bact.	8	32	49·04	1·97
		Act.	8	32	31·19	−0·03

Table V (*cont.*).

Soil no.	Addition		Number of sets (S)	S (n)	Total χ^2	Difference $\sqrt{2\chi^2} - \sqrt{2n-1}$
5	Mycelium of Trichoderma	Bact.	8	33	39·17	0·81
		Act.	8	33	*63·76*	*3·23*
5	Mycelium of Zygorhynchus	Bact.	9	38	48·38	1·18
		Act.	9	38	36·34	− 0·13
5	Mycelium of Act. griseus	Bact.	9	39	52·36	1·45
		Act.	9	39	39·47	0·11
5	Mycelium of Asp. fumigatus	Bact.	9	38	28·17	− 1·16
		Act.	9	38	51·68	1·51
5	Mycelium of Polyporus sp.	Bact.	10	47	52·70	0·63
		Act.	10	47	*25·65*	*− 2·48*
5	Protoplasm of Bac. megatherium	Bact.	7	31	31·44	0·12
		Act.	7	31	19·32	− 1·60

Sets with *S* (*n*) less than 30:   *P*

Soil no.	Addition		Number of sets (S)	S (n)	Total χ^2	P
5	Protoplasm of Bacterium sp.	Bact.	7	25	27·22	0·50–0·30
		Act.	7	25	20·01	0·80–0·70
5	Mycelium of Stachybotrys	Bact.	5	17	12·91	0·80–0·70
		Act.	5	17	12·87	0·80–0·70
5	Mycelium of Mycogone nigra	Bact.	6	24	32·90	0·20–0·10
		Act.	6	24	18·61	0·80–0·70
5	Mycelium of Coprinus sp.	Bact.	7	26	23·32	0·70–0·50
		Act.	7	26	20·81	0·80–0·70
5	Chitin	Bact.	5	23	20·12	0·70–0·50
		Act.	5	23	13·55	0·95–0·90
5	Keratin	Bact.	4	16	19·38	0·20–0·10
		Act.	4	16	6·33	*0·99–0·98*
4	Chitin	Bact.	4	15	*33·64*	*Below 0·01*
		Act.	4	15	8·10	0·95–0·90
4	Keratin	Bact.	3	12	*5·69*	0·95–0·90
		Act.	3	12	10·93	0·70–0·50
	Total	Bact.	—	1037	1181·97	3·09
		Act.	—	1037	916·43	− 2·72

Omitting the series with abnormal variation:

	Total	Bact.	—	942	1000·71	1·35
		Act.	—	786	717·72	− 1·75

The table shows that in the soils without addition of organic matter the values of χ^2 for both bacteria and actinomycetes were within the range of expectation in the individual series as well as in the total. In the series with organic matter the χ^2 for the bacteria were within the range of expectation in 23 cases out of 26; in the remaining 3, soil No. 2 with lime and farmyard manure, and soil No. 4 with chitin and with dried farmyard manure, there was a distinctly excessive variation. This appears very clearly in Table V, where all the values of χ^2 and *n* from series with organic matter are summed up and the difference calculated. If the three excessive series are omitted, the difference is reduced suffi-

ciently to come within the range of expectation. The figures for actinomycetes showed only 1 case of excessive variation (soil 5 with addition of *Trichoderma* mycelium), but 4 cases of subnormal variation (soil 1 with manure and with manure + straw, soil 5 with *Polyporus* mycelium, and soil 4 with chitin). None of the differences in the last 4 cases is much below − 2, and when the 5 abnormal series are omitted from the total, we obtain a result which is within the range of expectation. Thus, when this medium is used for microbial counts in connection with decomposition experiments with organic matter in soil, it is necessary to reckon with the possibility that the method may break down should a flora arise for which the medium is not suitable. Especially is this the case with the actinomycetes. For the ordinary soil flora, on the other

Table VI. *Values of χ^2 in different soils. Counts on mannite-asparagine agar.*

Soil no.	Addition		Number of sets (S)	S (n)	Total χ^2	Difference $\sqrt{2\chi^2} - \sqrt{2n-1}$
		Soils without organic matter.				
1	Nothing	Bact.	9	51	*80·61*	*2·63*
		Act.	9	51	35·44	− 1·63
5	Nothing	Bact.	13	62	59·03	− 0·22
		Act.	13	62	*39·03*	− *2·25*
	Total	Bact.	—	113	139·64	1·71
		Act.	—	113	*74·47*	− *2·80*
		Soils with organic matter.				
1	Farmyard manure	Bact.	9	52	61·42	0·91
		Act.	9	52	34·23	− 1·87
1	Do. + oat straw	Bact.	9	47	56·10	0·93
		Act.	9	47	36·03	− 1·16
5	Mycelium of *Asp. fumigatus*	Bact.	8	37	33·26	− 0·72
		Act.	8	37	41·05	0·53
5	Mycelium of *Polyporus* sp.	Bact.	9	43	39·46	− 0·34
		Act.	9	43	*18·48*	− *3·15*
Series with S (n) less than 30:						P
5	Mycelium of *Trichoderma*	Bact.	5	22	18·38	0·70–0·50
		Act.	5	22	22·79	0·50–0·30
5	Mycelium of *Zygorhynchus*	Bact.	6	27	24·39	0·70–0·50
		Act.	6	27	18·77	0·90–0·80
5	Mycelium of *Act. griseus*	Bact.	5	24	26·26	0·50–0·30
		Act.	5	24	25·81	0·50–0·30
5	Protoplasm of Bacterium sp.	Bact.	5	21	32·20	0·90–0·95
		Act.	5	21	21·47	0·50–0·30
						Difference
	Total	Bact.	—	273	291·47	0·79
		Act.	—	273	218·63	− 2·44
Omitting the series with abnormal variation:						
	Total	Act.	—	230	200·15	− 1·41

hand, the dextrose-casein agar seems well adapted to the counting of both bacteria and actinomycetes.

In Table VI the data obtained with mannite-asparagine agar from soils with and without organic matter have been treated 'in a similar way[1].

There is here an excessive variation in the bacterial counts in one of the soils without addition, but in all the others, as well as in the total (Table II), the variation is normal. The actinomycetes counts show subnormal variation in one of the soils without addition. Among the soils with organic matter, only one shows a definite subnormality; the others seem mostly to tend in the same direction, although the totals of χ^2 and n in soils with organic matter show a variation within the range of expectation, when the abnormal series is omitted. The mannite-asparagine agar thus seems under these conditions to resemble the dextrose agar, but whether it has more or less tendency to give subnormal variation than the latter medium can hardly be ascertained from the data available here.

The paper published by Waksman[8] provides sufficient data for the calculation of the distribution of χ^2 from counts of actinomycetes in soil.

Table VII. *Distribution of χ^2 in counts of actinomycetes*
(*Waksman's data*).

Sets with 10 plates			Sets with 8 plates		
χ^2	Expected	Observed	χ^2	Expected	Observed
0	0·5	1	0	0·5	0
2·088	0·5	1	1·239	0·5	0
2·532	1·5	0	1·564	1·5	1
3·325	2·5	3	2·167	2·5	4
4·168	5	5	2·833	5	4
5·380	5	3	3·822	5	2
6·393	10	11	4·671	10	7
8·343	10	8	6·346	10	7
10·656	5	6	8·383	5	4
12·242	5	5	9·803	5	1
14·688	2·5	1	12·017	2·5	11
16·919	1·5	4	14·067	1·5	1
19·670	0·5		16·622	0·5	0
21·666	0·5	2	18·475	0·5	8
Total	50	50		50	50

The medium used was dextrose-albumen agar[7], which gave slightly lower figures than casein agar. From Waksman's data 50 10-plate counts and 50 8-plate counts have been examined and the values of χ^2 calculated. The distributions are seen in Table VII.

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[1] Only 78 counts from soils 1 and 5 included here; the remaining 2 were from soil 2.

In the 10-plate series there is a good agreement between observation and expectation, but in the 8-plate series there is a marked tendency to excessive variation. These latter figures are all from one particular date of sampling and the effect may therefore be due to the temporary appearance of antagonistic organisms, depressing the development of actinomycetes on some of the plates (cf. Fisher, Thornton and Mackenzie (2)).

Rao and Subramanyan (3) compared a large number of agar media for counting soil actinomycetes. They finally adopted a medium containing starch and asparagine besides mineral nutrients, because parallel counts were more consistent on this than on any other medium tested by them. In their Tables XVI and XVII, they record the mean number of actinomycetes colonies $(\bar{x})$ and the variance $\left(V = \dfrac{S(x - \bar{x})^2}{n} \right)$ in 13 sets of counts on starch-asparagine agar and 10 sets on soil-extract agar. This enables us to calculate the values of $\chi^2 \left(= \dfrac{4V}{\bar{x}} \right)$, there being 5 parallel plates in each count, for actinomycetes counts on these two media. These results are recorded in Table VIII, which shows a very strong subnormality on starch agar, but on soil-extract agar a χ^2 which varies about a normal mean value.

Table VIII. *Counts of soil actinomycetes on starch agar and soil-extract agar in parallel soil samples (Rao and Subramanyan's data).*

Sample no.	Starch-asparagine agar			Soil-extract agar		
	$\bar{x}$	V	χ^2	$\bar{x}$	V	χ^2
1	38·6	9·3	0·96	30·3	51·0	6·73
2	40·2	3·7	0·37	36·2	16·5	1·64
3	38·5	2·3	0·24	37·3	19·0	2·09
4	36·6	3·3	0·36	39·0	51·5	5·28
5	39·2	5·7	0·58	43·0	150·5	14·00
6	38·8	5·7	0·59	36·0	12·0	1·50
7	38·8	6·7	0·67	30·0	5·5	0·73
8	38·0	5·0	0·53	38·2	85·4	8·94
9	39·2	6·7	0·68	37·2	77·5	8·33
10	40·2	3·5	0·35	34·8	31·6	3·59
11	39·4	10·3	1·05	—	—	—
12	38·4	4·5	0·47	—	—	—
13	39·2	11·2	1·14	—	—	—
Total χ^2	—	—	7·99	—	—	52·83
$S(n)$		52·00			40·00	
$\sqrt{2\chi^2} - \sqrt{2n-1}$		−6·15			+1·40	

In their Table XV, Rao and Subramanyan give the counts of actinomycetes colonies on individual plates (4 or 5 parallels) of starch agar

from .6 different soils. A calculation of χ^2 from these data shows (Table IX) a general tendency to subnormality, and a total of χ^2 which, as shown by Fisher's Table III, is quite outside the range of expectation. The tendency to subnormal variation is thus not limited to the one particular soil mentioned in Table VIII, and the medium therefore does not seem well adapted for counting soil actinomycetes.

Table IX. *Counts of actinomycetes on starch-asparagine agar in 6 soils (Rao and Subramanyan's data).*

Soil no. ...	I	II	III	IV	V	VI
$\bar{x}$	30·5	31·4	4·8	40·2	14·5	12·6
χ^2	0·36	0·61	0·58	1·69	0·90	0·41
n	3	4	4	4	3	4

$S(n) = 22.$ Total $\chi^2 = 4·55.$

SUMMARY.

1. Eighty counts of soil bacteria and actinomycetes, made on mannite-asparagine agar and dextrose-casein agar, showed that the latter medium gave significantly higher numbers of bacteria in 76 per cent. of cases, and significantly higher numbers of actinomycetes in 60 per cent. of cases.

2. On mannite-asparagine agar the values of χ^2 of the bacterial counts were as a whole in agreement with expectation. The counts of actinomycetes colonies on this medium showed a tendency to subnormal variation.

3. 357 counts of soil bacteria and actinomycetes on dextrose-casein agar gave distributions of χ^2, which mostly agreed with expectation. The bacterial counts showed a tendency to excessive, the actinomycetes counts to subnormal variation. These abnormalities seemed most liable to occur in soils where special microfloras had been accumulated as a result of the introduction of decomposable organic matter.

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